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Sarracenia flava

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Original Kodachromes by Mr. R. E. Stauffer. Republished from Wild Flower **27**: 32, 1951

(Accompanying "Natural Hybrids in the Genus *Sarracenia*," by C. R. Bell)

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A STUDY OF THE DISTRIBUTION AND LONGEVITY OF ADULT *TRICHINELLA SPIRALIS* IN IMMUNIZED AND NON-IMMUNIZED MICE

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There have been many reports on the distribution and longevity of adult *Trichinella spiralis* in the intestinal tract of various experimental hosts (Gursch, 1949). Of particular interest here are certain reports dealing with rats and mice (Tyzzer and Honeij, 1916; McCoy, 1931, 1932, 1940; Gursch, 1939, 1949; Rappaport, 1943; Rappaport and Wells, 1951; Larsh and Hendricks, 1949). While important information has been derived from such studies, comparisons are difficult, since certain important factors, such as age and sex of the host, size of infecting dose, etc., were not always controlled, and in many cases the studies were confined to either immunized or non-immunized hosts. There has been need, therefore, for a carefully controlled study dealing with both immunized and non-immunized animals. Aside from providing useful information on the distribution and longevity of adult *T. spiralis*, it was hoped that the present study in mice might reveal data of value in considering the resistance phenomena operative in this host-parasite combination.

Materials and Methods

Experimental Design

The mice of this study were of the same strain used in work reported previously from this laboratory (Larsh and Kent, 1949; Larsh and Hendricks, *l.c.*; and Hendricks, 1950). Eight-week-old mice, matched according to sex and approximate weight, were divided into experimental and control groups.

The stock strain of *Trichinella spiralis* is one that has been maintained in rats for a number of years. Larvae for each infection were obtained from rats which had been infected about eight weeks previously with 3,500 larvae. The methods for isolating and administering the larvae used in infections, and for recovering the adult worms, were essentially those described by Larsh and Kent (*l.c.*). Nevertheless, a full account is given below of the methods used in recovering and counting the adult worms, since the present experimental design differs slightly from that in our earlier work.

An infecting schedule known to produce demonstrable resistance (Hendricks, *l.c.*) was used for the experimental group. Three stimulating (immunizing) infections of 200 larvae each were given, three weeks apart, the last one being followed one month later by the challenging (test) infection of 200 larvae. Thus, all of the mice were about five months old at the time of the challenging infection. The controls received only the challenging infection. The viability of the larvae used for each stimulating infection was ascertained by determining the percentage development of adult worms in a few mice, viability controls, killed seven days after infection. In all cases, the percentage of development was within the usual range for this 200 dose. Persistency controls of the same age, given the same three stimulating infections but not the challenging infection were included to determine whether adult worms persisted until autopsy. Two such controls were autopsied along with each group of experimental and control mice in the experiment. Since no worms were recovered from the persistency controls, it seems safe to conclude that the worms recovered from the experimental mice were only those that developed from the challenging infection. With these explanations, it will not be necessary to consider further the viability and persistency controls.

All animals were denied food overnight before they were killed, to reduce the amount of material in the intestines. Four or five mice from each of the experimental and control groups were autopsied on the following numbers of days after the challenging infection: 3, 4, 5, 6, 7, 8, 11, 14, 17, 20, and 24. Therefore, at autopsy, the mice ranged in age from about 5.4 months to about 6.1 months. The small intestine of each mouse was divided into two equal parts, I (the first half) and II (the second half). Each half was placed in a glass funnel and cut longitudinally its entire length and then cut transversely into two-inch sections. The contents of each funnel were flushed with about 90 c.c. of 0.05% NaOH into a wide-mouthed, screw-cap jar. The large intestine, from the ileocecal valve to the anus, was cut longitudinally, gently washed free of fecal material, and placed in a separate jar with about 40 c.c. of the NaOH solution. Preliminary studies had revealed no worms in the fecal contents, so this material was discarded. After being refrigerated overnight at 45°F, the contents of the jars were shaken vigorously 100 times and the pieces of intestine discarded, since previous work had revealed no worms in them after such treatment. The fluid containing the worms, including the washings of the jar, was concentrated by centrifugation to about one c.c. Using a large glass slide, the total number of worms found in each section of intestine was determined by direct counts made under 30× magnification.

Statistical Methods

One of the objectives in the present study was to obtain quantitative information which might be helpful in understanding the resistance phenomena occurring in this host-parasite combination. This would necessitate first an examination of the distribution of adult worms among the three sites from day to day within each group. This type of comparison assumes relative importance

only when the distribution pattern in the immunized mice is different from that of the non-immunized animals. The statistical method, therefore, resolved itself into performing an analysis of variance such that a comparison of the adult counts, between the immunized and non-immunized animals, could be tested for significance. This comparison was to be made at each of the three sites where separate counts were obtained. Owing to the fact that differing numbers of animals were autopsied on the individual days and in the immunized and non-immunized groups, disproportionate numbers in the various subclasses resulted. A least squares solution was used to measure the effect of days and to test the difference between the immunized and non-immunized groups. This will be referred to later, in the discussion of the experimental results.

There was, in addition, other information of research value in the observations recorded. For example, it was helpful to have an accurate estimate of the day on which the difference between the two groups of animals, in the number of adults in the anterior portion of the small intestine, was a maximum. Also, it was valuable to have an accurate estimate of the day on which the distribution of adult *T. spiralis* reached a maximum count in the large intestine of the immunized and non-immunized animals. The first question was answered by testing the maximum difference between the immunized and non-immunized groups for significance against the adjoining differences on each side. The second question was answered by fitting mathematical curves to the observations. These distributions are presented graphically in a later section.

EXPERIMENTAL RESULTS

The average numbers of worms recovered from each group of experimental and control mice, autopsied from three to twenty-four days after the challenging infection, are shown in Table 1. The numbers of worms recovered from the anterior and posterior halves of the small intestine, the entire small intestine, the large intestine, and the entire intestine are listed. Percentages are given in terms of the ratio of the adults recovered to the total number of larvae administered in the challenging infection. As explained above, the absence of worms in persistency controls, given only the stimulating infections, allows the assumption that all of the worms recovered developed from the larvae administered in the challenging infection.

The recovery rate of adult worms from the control mice, from the third day through the eighth day after infection, shows that the majority of the worms was in the anterior half of the small intestine (from 46.0 per cent of the infecting dose on the fourth day to 68.9 per cent on the seventh day). During the same period large numbers also were recovered from the second half of the small intestine (from 16.1 per cent on the fourth day to 40.1 per cent on the eighth day). Only a small percentage of worms was found in the large intestine during this time (from 1.1 per cent on the seventh day to 5.5 per cent on the sixth day). The distribution of worms changed somewhat on the eleventh day. The number of worms in the posterior half of the small intestine had increased so that there were only a few worms fewer than in the anterior half (41.5 and 42.8 per cent, respectively),

TABLE 1

Showing the average number and per cent of infecting dose of adult T. spiralis *recovered from the intestinal tract of immunized and non-immunized mice following a test infection of 200 larvae*

DAYS AUTOPSIED AFTER CHALLENGED	IMMUNIZED MICE						NON-IMMUNIZED MICE					
	No. mice	Small Intestine			Large Intestine (% of dosage)	Entire Intestine (% of dosage)	No. mice	Small Intestine			Large Intestine (% of dosage)	Entire Intestine (% of dosage)
		First half (% of dosage)	Second half (% of dosage)	Total (% of dosage)				First half (% of dosage)	Second half (% of dosage)	Total (% of dosage)		
3	5	102.4	74.2	176.6	2.0	178.6	5	103.0	45.6	148.6	5.6	155.2
		(51.2)	(37.1)	(88.3)	(1.0)	(89.3)		(51.5)	(22.8)	(74.3)	(2.8)	(77.6)
4	5	78.6	47.4	126.0	7.0	133.0	4	92.0	32.3	124.3	3.0	127.3
		(39.3)	(23.7)	(63.0)	(3.5)	(66.5)		(46.0)	(16.1)	(62.1)	(1.5)	(63.6)
5	5	53.0	46.2	99.2	17.4	116.6	4	94.5	67.8	162.3	3.8	166.0
		(26.5)	(23.1)	(49.6)	(8.7)	(58.3)		(47.3)	(33.9)	(81.1)	(1.9)	(83.0)
6	5	43.8	72.2	116.0	28.0	144.0	4	104.3	63.5	167.8	11.0	178.8
		(21.9)	(36.1)	(58.0)	(14.0)	(72.0)		(52.1)	(31.8)	(83.9)	(5.5)	(89.4)
7	4	13.0	64.5	77.5	36.5	114.0	4	137.8	43.0	180.8	2.3	183.0
		(6.5)	(32.3)	(38.8)	(18.3)	(57.0)		(68.9)	(21.5)	(90.4)	(1.1)	(91.5)
8	5	31.2	59.2	90.4	16.2	106.6	4	104.3	80.3	184.5	3.8	138.3
		(15.6)	(29.6)	(45.2)	(8.1)	(53.3)		(52.1)	(40.1)	(92.3)	(1.9)	(69.2)
11	5	44.0	48.2	92.2	25.0	117.2	5	85.6	83.0	168.6	21.8	190.4
		(22.0)	(24.1)	(46.1)	(12.5)	(58.6)		(42.8)	(41.5)	(84.3)	(10.9)	(95.2)
14	5	9.6	51.4	61.0	15.2	76.2	5	6.0	48.2	54.2	69.0	123.2
		(4.8)	(25.7)	(30.5)	(7.6)	(38.1)		(3.0)	(24.1)	(27.1)	(34.5)	(61.6)
17	4	5.5	24.8	30.3	33.3	63.5	4	5.8	12.3	18.0	40.5	58.5
		(2.8)	(12.4)	(15.1)	(16.6)	(31.8)		(2.9)	(6.1)	(9.0)	(20.3)	(29.3)
20	5	1.2	5.2	6.4	8.0	14.4	4	1.3	3.5	4.8	25.3	30.0
		(0.6)	(2.6)	(3.2)	(4.0)	(7.2)		(0.6)	(1.8)	(2.4)	(12.6)	(15.0)
24	5	1.0	4.0	5.0	6.4	11.4	4	0.5	1.0	1.5	18.0	19.5
		(0.5)	(2.0)	(2.5)	(3.2)	(5.7)		(0.3)	(0.5)	(0.8)	(9.0)	(9.8)

while the number in the large intestine also had increased greatly (10.9 per cent). It would seem, therefore, that the distribution of worms, which had been essentially the same irrespective of the length of the period of infection, was changing and the worms were beginning to be eliminated. This trend continued throughout the remainder of the experiment, with smaller numbers in the anterior half of the small intestine than in either the posterior half or the large intestine.

The percentages of worms in the entire small intestine of the controls showed the greatest drop between the eleventh and the fourteenth days, from 84.3 to 27.1, respectively, while the percentages in the large intestine showed the greatest increase, from 10.9 to 34.5 per cent of the total dose administered, during this period. There is evidence here that many of the worms eliminated from the small intestine pass directly out of the body, since the numbers in the large intestine, although increased, were not great enough to account for those lost from the small intestine. It is also important to note that, of the percentage found in the entire small intestine on the fourteenth day (27.1), most of the worms (24.1 per cent) were in the posterior half. These observations have a bearing on resistance to be discussed below.

The percentage of worms in the entire intestine of the controls remained quite constant through the eleventh day (varying from 63.6 on the fourth day to 95.2 on the eleventh day after infection). After this time, the worms were eliminated in large numbers, to the lowest level of 9.8 per cent on the twenty-fourth day. It is worth noting that 9.0 per cent of these worms were in the large intestine and only 0.8 per cent in the entire small intestine.

As shown in Table 1, the recovery rate of adult worms from the experimental mice, which had been immunized with three stimulating infections, differed greatly from that of the control mice. On the third and fourth days after infection, larger numbers of worms were found in the first half of the small intestine (51.2 and 39.3 per cent, respectively) than in the second half (37.1 and 23.7 per cent, respectively), and there were relatively few adults in the large intestine (1.0 and 3.5 per cent, respectively). On the fifth day, there was a striking reduction in the number of worms in the small intestine and there were approximately equal numbers in both halves (26.5 and 23.1 per cent, respectively), whereas an increased percentage, compared with the previous day, occurred in the large intestine (8.7). From the sixth day throughout the remainder of the experiment a reversal in distribution occurred and greater numbers of worms were recovered from the posterior half of the small intestine (from 2.0 per cent on the twenty-fourth day to 36.1 per cent on the sixth day) than from the anterior half (from 0.5 per cent on the twenty-fourth day to 21.9 per cent on the sixth day). During most of this period, the percentage of worms recovered from the large intestine remained rather high. It first dropped to a low level, 4 per cent, on the twentieth day, but by this time the entire small intestine contained only 3.2 per cent of the infecting dose.

The percentage of worms in the entire small intestine of the experimental mice first showed a striking reduction on the fifth day (49.6). Except for the sixth day, when the percentage rose to 58.0, this downward trend continued through-

out the experiment, reaching its lowest level, 2.5 per cent, on the twenty-fourth day. The percentage in the large intestine fluctuated greatly during this period and did not show, to the same degree as in the controls, the tendency to increase as the percentages in the small intestine decreased.

The percentage of worms in the entire intestinal tract of the experimental mice remained rather high through the eleventh day (from 53.3 per cent on the eighth day to 89.3 on the third day). On the fourteenth day there was a considerable reduction in the total number of worms (38.1 per cent), a trend which continued throughout the rest of the experiment, reaching its lowest level, 5.7 per cent, on the twenty-fourth day.

DISCUSSION

These data reveal several important differences in the distribution and longevity of adult *T. spiralis* in the non-immunized and immunized mice. During the first eight days of infection, the non-immunized controls harbored the majority of their worms in the anterior half of the small intestine. This agrees with previous findings in old mice killed five days after infection (Larsh and Hendricks, 1949). It was not until the eleventh day, in the present case, that a trend was shown toward the occurrence of larger numbers of worms in the posterior half. On the other hand, the immunized mice, as expected from earlier work, showed evidence of this trend after five days. In order to test the significance of the differences, in the anterior half, between the immunized and the non-immunized mice, the data were divided into two sections, respectively, for the periods of 3–11 days and 14–24 days following infection. This was done because there was clear-cut evidence of a strikingly large falling-off in adult worms in both groups following the eleventh day. An analysis of the results as one intact group would have violated the assumption of a homogeneous experimental error. The analysis of variance for the 3–11 days group is presented in Table 2.

It can be seen from Table 1, and in the analysis of variance (Table 2), that the immunized mice had in the anterior half of the small intestine a significantly lower count of adult worms after the third day following infection, and that this was manifest throughout the 3–11 day period. For both immunized and non-immunized mice, the effect of the day on which the worms were counted was also significantly variable. This reduction in the number of worms in the day-to-day count was primarily due, however, to the decrease among the immunized animals. This is verified by the significance at the 1% level of the interaction of days with immunization. In fact, the difference between immunized and non-immunized animals varied from 0.6 to 124.8 worms. The pattern of this variation was such that the difference started at 0.6, systematically reached a maximum of 124.8 at the end of the seventh day, and then proceeded to fall off to the vanishing point during the following week. Thus, in studies on acquired resistance using mice of this age infected with 200 larvae, comparisons of immunized animals and controls should be made approximately seven days after the challenging infection.

The analysis of variance for the anterior portion of the small intestine for the

period of 14–24 days is not shown in this article. It has been computed, however, and the only effect of significance was that of days. This confirmed the observation that after the eleventh day, both groups of mice appeared to have similar counts which were gradually eliminated.

The reduction of worms in the anterior half of the small intestine in both groups of mice probably was due to the action of defensive mechanisms. In the controls, infected for the first time, the mechanisms may form part of the natural defense against these worms, but it is likely that mechanisms associated with acquired resistance had become active within eleven days. In any event, the mechanisms operative in the immunized mice functioned more rapidly and showed an effect on the distribution of worms approximately six days sooner than in the controls.

TABLE 2

Analysis of variance of counts in the anterior half of the small intestine, 3–11 days following infection

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
Total	63	94,377.61		
Immunization (adjusted for days)	1	37,671.58	37,671.58	78.90**
Days (unadjusted)	6	10,845.40		
Days (adjusted for immunization)	6	10,211.22	1,701.87	3.56**
Immunization (unadjusted)	1	38,305.76		
Interaction of days and immunization	6	21,986.18	3,664.36	7.67**
Experimental error	50	23,874.45	477.49	

** Significant at 1% level.

In the distribution of worms in the posterior half of the small intestine, entirely different results were obtained. The analysis of variance for adult counts in the posterior half of the small intestine is presented in Table 3. It can be seen that the immunized mice did not differ significantly from the non-immunized group. Thus, the reduction of worms in the anterior half by the fifth day, in the experimentals, did not cause a corresponding increase in the posterior half. Days were significant in this analysis. This was caused by the great loss of worms after the fourteenth day. For the first fourteen days after infection, however, the number of worms in this region did not vary significantly in either group separately, or when both groups were combined. It can also be seen in the analysis of variance that despite the fact that immunization made little difference, the interaction of days with immunization was significant at the 1% level. This was caused by the fact that although there was no consistent favoring of one group or the other throughout the entire period, the magnitude of the differences between the two groups varied significantly from day to day.

It is worth noting that the experimentals harbored more worms than the

controls, following the seventeenth day, although this difference was not statistically significant. It is, therefore, clear that the resistance acquired by the experimentals from prior immunizations, which was effective so promptly in eliminating worms from the anterior half of the small intestine, did not manifest itself in eliminating worms from the posterior half at a rate faster than that in controls. This seems to suggest that in these old mice the most important mechanisms of resistance to adult *T. spiralis* are localized in the first half of the small intestine. However, in young mice, which have a much faster intestinal emptying time, the second half of the small intestine might be more important, since the majority of worms have been observed in that region (Larsh and Hendricks, 1949).

TABLE 3

Analysis of variance of counts in the posterior half of the small intestine

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
Total	99	90,866.75		
Immunization (adjusted for days)	1	32.73	32.73	<1 N.S.
Days (unadjusted)	10	54,964.34		
Days (adjusted for immunization)	10	54,988.46	5,498.85	15.98**
Immunization (unadjusted)	1	8.61		
Interaction of days and immunization	10	9,023.73	902.37	2.62**
Experimental error	78	26,845.95	344.18	

** Significant at 1% level.

In the distribution of worms in the large intestine, the experimentals showed the largest percentage (18.3) on the seventh day, whereas the peak for the controls (34.5) was not reached until fourteen days. Before testing the difference in the distribution of worms in the large intestine, it was evident that the situation had been the reverse of the one observed in the anterior portion of the small intestine. The immunized mice had a larger count of worms until the eleventh day, after which the control mice had the greater number. To conform with the prior analysis of variance calculated for the first half of the small intestine, the data were again separated into 3–11 days and 14–24 days. The analysis of variance of the worms in the large intestine for the 3–11 day period is given in Table 4.

It can be seen from the analysis of variance that all three effects, immunization, days, and their interaction, were significant. This means that the immunized mice consistently had more worms than the controls during the 3–11 day period. The difference in number between the two, however, was not uniform from day to day. In fact, the excess among the immunized group rose from a small number (or none) to a maximum in one week (34.2 worms) and then declined again. Thus, seven days following infection, the large intestine of the

immunized mice showed a maximum excess over the non-immunized group whereas on the same day the anterior half of the small intestine indicated the exact reverse.

For the period from 14–24 days in the large intestine all effects were again significant at the 1% level. Thus, a significantly greater number of worms was found in the large intestine of the non-immunized group during this period. The maximum difference (53.8) occurred on the fourteenth day following infection. Coupling this with the finding for the previous period, 3–11 days, it is clear that in immunological experimentation, the choice of the correct time of autopsy is of prime importance. If the experimenter should examine the large intestine too early, the immunized mice would appear in a less favorable light.

TABLE 4

Analysis of variance of counts in the large intestine from 3–11 days

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F-RATIO
Total	63	9,945.86		
Immunization (adjusted for days)	1	1,811.72	1,811.72	32.15**
Days (unadjusted)	6	3,267.73		
Days (adjusted for immunization)	6	3,338.05	556.34	9.87**
Immunization (unadjusted)	1	1,741.40		
Interaction of days and immunization	6	2,049.16	341.53	6.06**
Experimental error	50	2,817.25	56.35	

** Significant at 1% level.

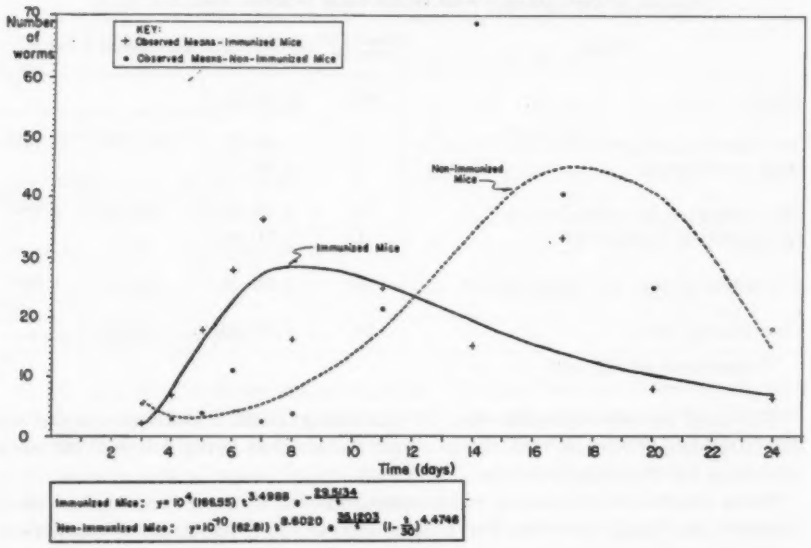
This may be seen in another way by examining Graph 1. Both groups showed similarly shaped curves, with the peak for the controls being delayed one week later than for the experimentals.

These results are interesting when compared with those of the distribution of worms in the small intestine. For both groups of mice, the greatest numbers of adults were found in the large intestine soon after the greatest loss occurred in the first half of the small intestine (between 5–7 days for the experimentals, and 11–14 days for the controls). This suggests that most of the worms eliminated from the first half of the small intestine pass quickly into the large intestine with little time, if any, being spent in the second half of the small intestine. This is supported by the numbers found in the posterior half of the small intestine, as there was little change noted during and following these periods; in fact, a reduction occurred after six days in the experimentals and eleven days in the controls. Thus, most of the worms in the second half of the small intestine probably were distributed there soon after infection. If this were the case, then, in both groups of mice, the slower elimination of worms from that region, as compared with that from the first half, must be due either to the smaller numbers

involved or to the fact that this region is less suitable for the operation of the defensive mechanisms. The latter seems most likely, when considering that the experimentals had received three infections previously and were even less able than the controls to eliminate worms from this region. As mentioned above, the region of greatest importance in resistance may be related to the age of the mice. Further studies are in progress which were designed in attempts to learn more about this suggested localization of the resistance. If it could be proved that a limited area of the small intestine is most important in this respect, it might facilitate studies of the mechanisms involved in the resistance.

GRAPH I

Number of Adult T. spiralis Recovered from the Large Intestine of Immunized and Non-Immunized Mice.



SUMMARY

This study of the distribution and longevity of adult *Trichinella spiralis* included immunized and non-immunized mice five months old at the time of the test infection with 200 larvae. Comparisons were made at intervals within a period of 3–24 days after infection.

As expected from previous work, the immunized mice showed a striking reduction in numbers of worms in the anterior half of the small intestine within five days after infection, whereas this period was extended to fourteen days in the controls. The maximum difference between the two groups occurred on the seventh day. Thus, in experiments with similar design, this is the post-infection

period recommended for comparing the two groups of mice. Both groups of mice failed to eliminate many worms from the posterior half of the small intestine until seventeen days after infection, at which time the immunized mice harbored more worms than the controls. In the distribution of worms in the large intestine, both groups showed similar curves, but the peak for the controls was one week later (fourteen days) than for the immunized mice. Thus, at seven days, the number of worms in the immunized mice showed a maximum excess over that of the non-immunized group, whereas on that day the number in the anterior half of the small intestine showed the exact reverse. In both groups, the peak of worms in the large intestine followed shortly upon the sudden loss of worms from the anterior half of the small intestine.

There is discussion of these results which seem to indicate that, in the mice studied, the anterior half of the small intestine is the site most active in acquired resistance. Further studies are in progress which were designed in attempts to learn more about this suggested localization of the resistance.

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STUDIES IN MICE ON THE DUAL ANTIBODY BASIS OF ACQUIRED RESISTANCE TO *TRICHINELLA SPIRALIS**

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It is well known that many laboratory animals develop acquired resistance to the nematode, *Trichinella spiralis*, following one or more stimulating (immunizing) infections. Such resistance has been demonstrated, in most cases, by a reduction in the numbers of parasites developing from a later, challenging (test) infection, as compared with the numbers in control animals not previously infected (McCoy, 1931, 1935; Roth, 1939; Culbertson, 1942; Larsh and Kent, 1949).

Specific antibodies are elaborated as a result of infection with this parasite. These substances have been detected in man and various experimental animals by certain routine serological techniques (Bachman, 1929; Culbertson and Kaplan, 1938; Melcher, 1943; Hendricks, 1950). In mice given repeated infections there is a direct relation between the ability of the animals to eliminate worms from the small intestine and the concentration of serum antibodies (Hendricks, *l.c.*). It has been generally agreed that this resistance operates against the adult worms in the intestine to interfere with the reproduction potential of the females and to reduce the longevity of both sexes. It was natural to assume, therefore, that the only antibodies formed were those against the adults. However, an outstanding contribution was made by Oliver-Gonzalez (1940, 1941) when he discovered that there are at least two different antibodies, one operating primarily against the adults, the other against the larvae. Evidence was also presented supporting the theory that many different antibodies are elaborated during an infection, some against antigens common to both adults and larvae.

Because of its importance in understanding the mechanism of resistance to *T. spiralis*, it was decided to study further the dual antibody basis of this resistance. As brought out in the work of Oliver-Gonzalez, mentioned above, it is difficult to make such studies in animals infected in the usual way, since both stages of the parasite, adults and larvae, develop in the same host. It was decided, therefore, to refine the technique so that only one stage would be involved. Thus, larvae for infections were irradiated to prevent sexual maturity. In this way, resistance resulting from "adult" only infections could be compared with the usual type involving both stages. Following 1–6 stimulating infections, com-

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parisons were made of the resistance produced by the two types of infections and the resulting adult and larval antibody titers.

MATERIALS AND METHODS

The mice used in this study were of the same strain as those used in earlier studies reported from this laboratory (Larsh and Kent, 1949; and others). For ease in handling large numbers of mice and in tabulating and analyzing the data, the experiment was divided into six series. Each series of the experiment contained three groups of 20 mice each; the experimental group, the infected-control group, and the non-infected-control group. For clarity, infections given in an attempt to produce acquired resistance will be called stimulating infections, and the final infection, given to demonstrate the resistance developed, will be called the challenging infection. All of the animals for each series were males, six weeks old at the beginning of the experiment, and were divided according to approximate weight into the respective groups.

The stock strain of *T. spiralis* has been maintained in rats for a number of years. The methods for isolating the larvae for infection and for determining the number of adult worms in the small intestine are essentially the same as those described in earlier work (Larsh and Kent, *l.c.*). The freed larvae to be used for infections were concentrated into the bottom of a 30 c.c. conical centrifuge tube and resuspended in 20 c.c. of normal saline. This was mixed thoroughly and divided into equal portions. One of these portions was transferred to a small flat pyrex dish (diameter 2 in., height 0.5 in.) and irradiated with 3,700 roentgen units.* The concentration of both the irradiated and untreated larvae in their respective tubes was fixed at 200 per 0.1 c.c. by repeated counts and adjustments, either by dilution or concentration. Both standardization and administration of the dosages were made by the use of a blunted, slightly curved, 16 gauge needle attached to a 0.25 c.c. syringe.

The use of the precipitin test and the preparation of the larval antigen was described in a previous paper (Hendricks, 1950). Preparation of the adult antigen was similar to that of the larval antigen, but the collection of adults deserves comment. The sexually immature worms used were obtained from rats which had been infected with irradiated larvae (3,700 r) seven days prior to collection. After the rats were killed the small intestine was removed and slit longitudinally. Lengths of the intestine were placed in a Petri dish with a small amount of saline, and the mucosa scraped with the end of a glass slide. The remaining portion of the gut was rinsed in saline and discarded. The shreds of mucus were finely divided by rubbing them against the glass with the fingers. This suspension was placed on a filter paper in a Buchner funnel and, with a slight amount of suction, the excess saline was removed. The filter paper was then inverted in a Baerman-like apparatus (Figure 1). The apparatus consisted of a six-inch funnel with a one-inch stem which was stoppered with a cork. A circle of galvanized wire (one-fourth-inch mesh) was placed in the top of the funnel and just covered with

* Irradiation of the larvae was performed at the X-ray therapy department of Duke University Hospital through the courtesies of Dr. James R. Reeves and staff.

warm (37–42° C) saline. The filter paper containing the adults and the mucus shreds was inverted on the wire support and most of the worms were found to be freed within five minutes. In a short time the adults were found settled into the narrow stem of the funnel, and could easily be removed with a pipette. Worms thus collected were pooled, washed free of host protein and rapidly frozen. The steps in the preparation of antigen from this material, and the use of the antigen in the precipitin tests, are the same as those described previously for larvae (Hendricks, *l.c.*).

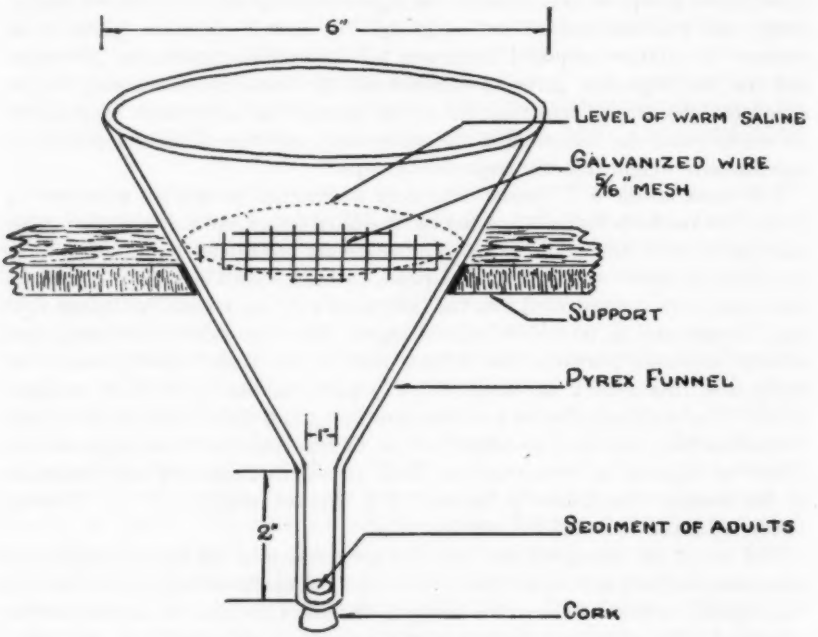

FIG. 1. Modified Baerman apparatus.

EXPERIMENTAL PROCEDURE AND RESULTS

In Series 1, the experimental animals were given a stimulating infection of 200 irradiated larvae, and the infected-controls were given a similar number of non-irradiated (untreated) larvae. To check the viability of the larvae used in these infections, four additional mice were included in each group, which were killed six days later. As the number of adult worms present in these "viability control" mice was within the range expected, the findings will not be listed. These mice were not necessary in the remaining series of the experiment, as the non-infected-controls, killed six days post-infection in all cases, served this purpose. In addition to viability controls in this first series, five mice were included in each infected group to serve as "persistency" controls. These were

given the stimulating infection but not the challenging infection, and were killed with the groups mentioned above, 6 days after the challenging infection, *i.e.* 27 days after they were infected. Since these persistency controls received only the stimulating infection, the number of worms recovered from them indicated the degree of persistency. Such controls were not necessary in the remaining series of the experiment.

Three weeks after the stimulating infection was given to the experimental mice and the infected-control mice, the mice of each group were separated into two subgroups of 10 mice each. The mice of one subgroup of each group were given the challenging infection with 200 irradiated larvae. The mice of the other subgroup were given the challenging infection with 200 non-irradiated larvae. Six days later, all of the animals were bled, then killed. The number of adult worms present in the small intestine was determined by direct counts. The degree of acquired resistance, developed by the experimentals and the infected-controls as the result of the one stimulating infection, was measured by the number of adult worms recovered, as compared with the number recovered from the non-infected-controls. The antibody titer of the various groups of mice was determined by the precipitin test.

To determine the effect of repeated stimulating infections on the development of resistance and the antibody titer, five additional series were included in the experiment. Since the viability and persistency controls were not necessary, as explained above, these series differed from Series 1 only in the number of stimulating infections. Thus, in Series 2, 3, 4, 5, and 6, the experimental and infected-control groups were given 2, 3, 4, 5, and 6 stimulating infections, respectively, before their resistance was challenged by a final infection. In all cases, the non-infected-controls were given only the final infection. Since the latter were not stimulated, the number of worms developing in them shows the degree of natural resistance of mice of this age. Thus, the difference between the number of worms in these controls and the number in mice given the stimulating infections can be attributed to the action of acquired resistance.

The results of all six series are summarized in Table 1 (adult worm counts) and Table 2 (precipitin titers). These results may be seen another way by examining Figure 2 (adult worm counts), Figure 3 (precipitin titers of the experimental mice) and Figure 4 (precipitin titers of the infected-control mice).

In Series 1 (Table 1), the experimental mice harbored significantly fewer worms than the infected-controls (average 142.2 and 154.2, respectively) but neither group averaged significantly fewer worms than the non-infected-controls (145.5). The persistency controls for the experimentals, given only the stimulating infection with irradiated larvae, harbored only 3 worms, whereas those for the infected-controls, stimulated with non-irradiated larvae, had 18 worms. Therefore, the higher number of worms in the infected-control mice (154.2) may be accounted for by the persistency of some adults from the first infection, 27 days before autopsy. The type of larvae used in the challenging infection, *i.e.*, whether irradiated or non-irradiated, showed no significant effect on the numbers of adults recovered.

TABLE 1

A summary of Series 1–6, the average number of adult T. spiralis *recovered from the mice of the three groups of each series, following a challenging infection with 200 irradiated or non-irradiated larvae*

SERIES NUMBER	EXPERIMENTALS			INFECTED-CONTROLS			NON-INFECTED CONTROLS		
	Stimulating infections with 200 irradiated larvae			Stimulating infections with 200 non-irradiated larvae			Without stimulating infections		
	Challenging infection with irradiated larvae	Challenging infection with non-irradiated larvae	Average number adults	Challenging infection with irradiated larvae	Challenging infection with non-irradiated larvae	Average number adults	Infected with irradiated larvae	Infected with non-irradiated larvae	Average number adults
1	144.2	140.1	142.2	147.3	161.1	154.2	141.1	149.9	145.5
2	120.4	111.2	115.8	84.3	82.9	83.6	132.2	129.4	130.8
3	74.8	70.3	72.6	64.7	68.3	66.5	136.6	140.0	138.3
4	69.6	61.3	65.5	50.5	46.6	48.6	125.0	116.6	120.8
5	61.1	63.2	62.2	50.1	36.1	43.1	128.8	125.3	127.1
6	27.0	28.5	27.8	30.1	24.1	27.1	125.8	122.0	123.9

In Series 2, significantly fewer worms were recovered from both the experimentals (115.8) and infected-controls (83.6) than from the non-infected-controls (130.8). Likewise, the average numbers of worms recovered from the experimentals and infected-controls were significantly smaller than from similar groups in Series 1.

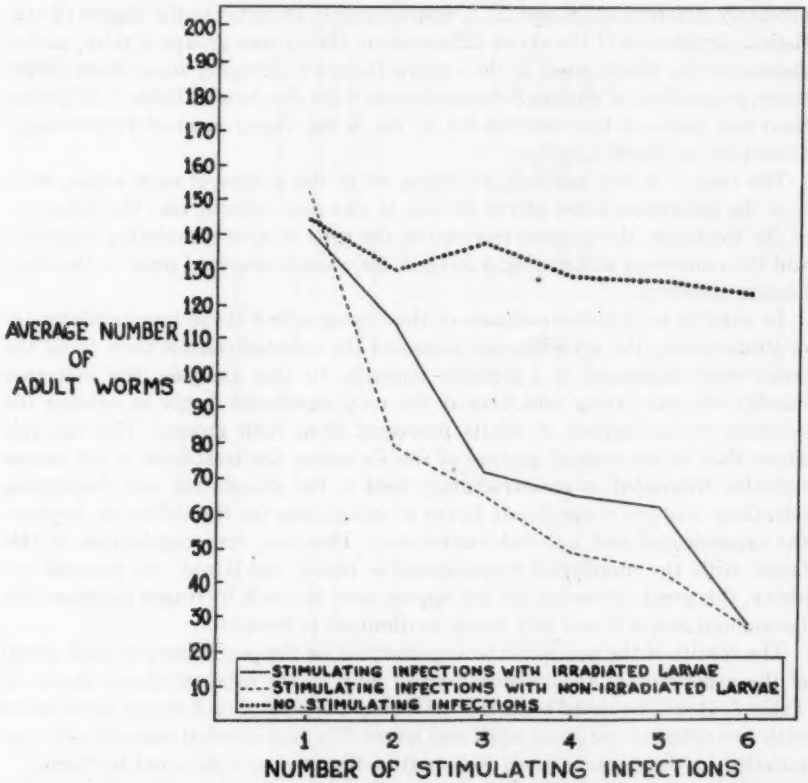

FIG. 2. The average number of adult worms recovered from the challenging infections of 200 larvae given to three different groups of mice: (1) experimental mice, (2) infected-control mice, and (3) non-infected-control mice. (Challenged following graded stimulating infections)

In Series 2, 3, 4, 5, and 6, the trend shown in Series 1, toward reduced numbers of worms in the experimentals and infected-controls, as compared with the numbers in non-infected-controls, was continued. The average numbers of worms in the experimentals ranged from 72.6 in Series 3 to 27.8 in Series 6, while the range for the infected-controls was from 66.5 in Series 3 to 27.1 in Series 6. By comparison, the average number of worms recovered from the non-infected-

controls remained about the same, from 138.3 in Series 3 to 120.8 in Series 4. It is also important to note that, in general, the average numbers from the experimentals and infected-controls of each series were significantly smaller than those from the respective groups in the preceding series.

In Series 2–5, the experimentals harbored significantly more worms than the infected-controls, but in Series 6, the numbers for the two groups were not significantly different (27.8 and 27.1, respectively). To indicate the degree of statistical significance of the above differences in the various groups of mice, and to determine the effectiveness of the various factors in bringing about these differences, an analysis of variance was performed with the data of Table 1. (Calculations and tables of this analysis are on file in the Department of Parasitology, University of North Carolina).

The results of this analysis, including all of the groups of each series, show that the differences listed above are due to two main effects, *viz.*, the difference in the treatment the animals received at the time of each stimulating infection, and the number of stimulating infections the animals received prior to the challenging infection.

In order to get a better estimate of the average effect of the increased number of stimulations, the experimental mice and the infected-control mice of all the series were considered in a separate analysis. In this analysis, the increased number of stimulating infections is the only significant factor in causing the decrease in the number of adults recovered from both groups. This analysis shows that in the over-all picture of the six series, the treatment of the larvae (whether irradiated or non-irradiated) used in the stimulating and challenging infections was not a significant factor in accounting for the difference between the experimental and infected-control mice. However, the combination of this factor with the number of stimulations is highly significant. As pointed out above, this great difference did not appear until Series 2, increased progressively throughout Series 5, and had begun to diminish in Series 6.

The results of the precipitin tests performed on the pooled sera of each group of the experimental and infected-control mice in the experiment are shown in Table 2. It will be noted from the table that the sera of each group were tested with two different antigens, adult and larval. The non-infected-controls were not included in these comparisons since antibodies were not detected in them.

In Series 1, the experimental mice challenged with irradiated larvae showed a precipitin titer of 1:5 with both adult and larval antigen, while those challenged with non-irradiated larvae showed a 1:10 titer with both antigens. The reverse was true in the infected-controls, in which those mice challenged with irradiated larvae showed a 1:10 titer with both antigens, while the titers of those challenged with non-irradiated larvae was 1:5. Thus, titers of the experimental mice were not significantly different from those of the infected-controls.

In Series 2–6, the adult and larval antibody titers of the experimental mice were similar whether challenged with irradiated or non-irradiated larvae. There was a progressive increase in the average adult antibody titer from 1:80 in Series 2 to 1:1920 in Series 6. The average larval antibody titer of these mice, while

TABLE 2

A summary of Series 1–6, the adult and larval precipitin titers of experimental and infected control mice of each series given a challenging infection with 200 irradiated or non-irradiated larvae

SERIES NUMBER	EXPERIMENTALS						INFECTED CONTROLS					
	Stimulating infections with 200 irradiated larvae						Stimulating infections with 200 non-irradiated larvae					
	Challenging infection with irradiated larvae		Challenging infection with non-irradiated larvae		Ave. for adult antigen	Ave. for larval antigen	Challenging infection with irradiated larvae		Challenging infection with non-irradiated larvae		Ave. for adult antigen	Ave. for larval antigen
	Adult antigen	Larval antigen	Adult antigen	Larval antigen			Adult antigen	Larval antigen	Adult antigen	Larval antigen		
1	1:5	1:5	1:10	1:10	1:7.5	1:7.5	1:10	1:10	1:5	1:5	1:7.5	1:7.5
2	1:80	1:40	1:80	1:40	1:80	1:40	1:80	1:40	1:80	1:40	1:80	1:40
3	1:320	1:80	1:320	1:80	1:320	1:80	1:320	1:320	1:320	1:320	1:320	1:320
4	1:1280	1:320	1:1280	1:320	1:1280	1:320	1:1280	1:640	1:1280	1:640	1:1280	1:640
5	1:1280	1:320	1:1280	1:320	1:1280	1:320	1:1280	1:1280	1:1280	1:1280	1:1280	1:1280
6	1:2560	1:320	1:1280	1:320	1:1920	1:320	1:2560	1:1280	1:2560	1:2560	1:2560	1:1920

less than that of the adult antibody, also showed a progressive increase from 1:40 in Series 2 to its maximum of 1:320 in Series 4.

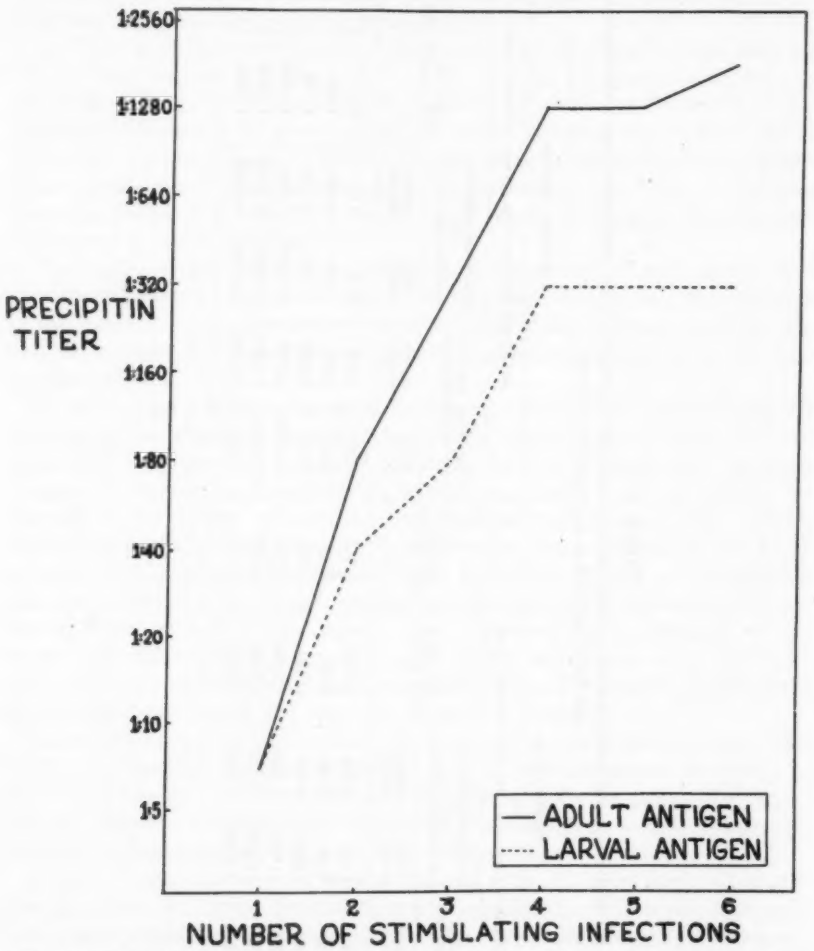

FIG. 3. The precipitin titers for the experimental mice of the six series given various numbers of stimulating infections with irradiated larvae. (Sera tested with both adult and larval antigen)

The infected-control mice of Series 2–6 showed similar adult and larval antibody titers whether they were challenged with irradiated or non-irradiated larvae. There was a progressive increase in the averages of both the adult and larval antibody titers. The average adult antibody titer increased from 1:80 in Series 2

to 1:1280 in Series 4 and 5, and further increased to 1:2560 in Series 6. The average larval antibody titer increased by two-fold dilutions through Series 5,

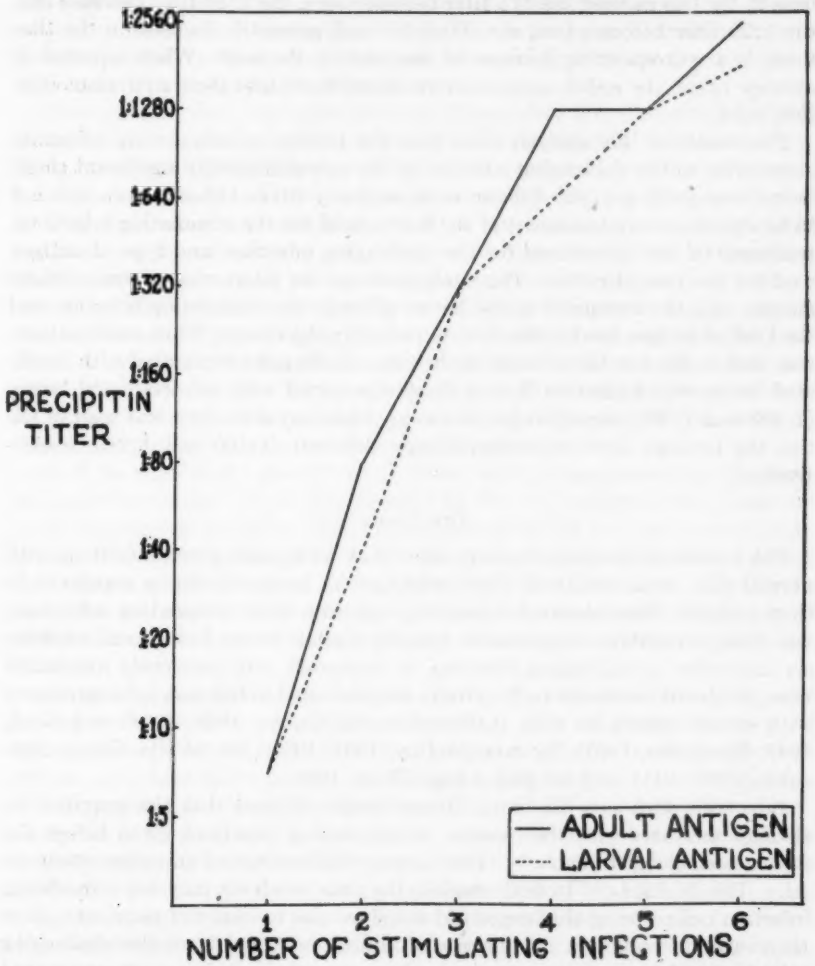

FIG. 4. The precipitin titers for the infected-control mice of the six series given various numbers of stimulating infections with non-irradiated larvae. (Sera tested with both adult and larval antigen)

from 1:40 in Series 2 to 1:1280 in Series 5. It further increased to 1:1920 in Series 6.

The analysis of variance for the precipitin titers was performed as for the adult counts. The titers given in Table 2 were coded in such a manner that the

distribution is similar to a normal curve. This is done by dividing each of the dilutions by 5 (the lowest antigen dilution), and then taking the logarithm to the base 2. By this method the 1:5 titer becomes zero, the 1:10 titer becomes one, the 1:20 titer becomes two, etc. Thus for each geometric increase in the titer there is a corresponding increase of one unit in the scale. When reported as average titers, the coded numbers were transformed into their equivalent dilution units.

The results of this analysis show that the number of stimulating infections given prior to the challenging infection is the only statistically significant single factor contributing to the differences in antibody titers. Other factors seen not to be significant are treatment of the larvae used for the stimulating infections, treatment of the larvae used for the challenging infection, and type of antigen used for the precipitin test. The analysis shows the interaction of two of these factors, *viz.*, the treatment of the larvae given in the stimulating infection and the kind of antigen used in the test, to be highly significant. When adult antigen was used in the test the average of the titers of the mice stimulated with irradiated larvae was similar to that of those stimulated with non-irradiated larvae (1:293 and 1:307, respectively). However, when larval antigen was used in the test the average titers were significantly different (1:100 and 1:240, respectively).

DISCUSSION

The results of the present study show that white mice given infections with normal (*i.e.*, non-irradiated) *Trichinella spiralis* larvae develop a resistance to that parasite. This acquired resistance, resulting from stimulating infections, was demonstrated by a reduction in number of adult worms in the small intestine six days after a challenging infection, as compared with previously uninfected mice. Acquired resistance to *T. spiralis* demonstrated in this way is in agreement with earlier reports for mice (Culbertson and Kaplan, 1938; Larsh and Kent, 1949; Hendricks, 1950), for rats (McCoy, 1931, 1935), for rabbits (Oliver-Gonzalez, 1940, 1941), and for guinea pigs (Roth, 1939).

Infections with non-irradiated larvae further showed that the acquired resistance increases with the number of stimulating infections given before the mice are challenged (Table 1). This agrees with findings of an earlier study on mice (Hendricks, *l.c.*). In both studies, the mice receiving only one stimulating infection before being challenged did not show this evidence of resistance, since there was no reduction in numbers of adults recovered from the challenging infection. In fact, in the present study there were more adult parasites recovered from these mice than from the controls not given the stimulating infection. This can be accounted for by the presence of some adults which persisted from the stimulating infection given 27 days before autopsy. In addition to these results, confirming earlier findings, new information was obtained which sheds further light on the dual antibody basis of resistance to *T. spiralis*.

Irradiation of the larvae did not affect their infectivity for mice, since the number of adult worms that were established in the small intestine, after an initial infection, was about the same as that from an infection with non-irradiated

larvae. This was expected from the results obtained by Levin and Evans (1942). These investigators showed, in addition, that rats given one infection with irradiated larvae developed acquired resistance. This differs from the present case, since there was no demonstrable resistance in the mice. This discrepancy is probably related to the animal host used, since the findings for the mice are in agreement with earlier results (Hendricks, *l.c.*).

Having shown that irradiation with this dosage does not affect the infectivity of the larvae, it is interesting that, in general, the experimental mice given stimulating infections with such larvae did not produce as great a degree of acquired resistance as the infected-controls given non-irradiated larvae. The present data offer no explanation for this difference. However, by this irradiation technique two phases of the infection are prevented, *viz.*, sexual maturity and larviposition. These effects were verified by the absence of larvae in the digested carcasses of mice killed 30 days after infection. The work of Oliver-Gonzalez (1941) suggests that the immature larvae in the uterus of the female worm resemble the adult worm in antigenicity and gradually, over a period of 20 days, acquire new antigenic characteristics peculiar to mature larvae. Therefore, in the absence of developing larvae in the experimental mice one might expect less antigenic stimulation, hence less acquired resistance to reinfection.

All of the mice in the present study, which were given stimulating infections, elaborated antibodies which were detected by the ring-precipitin test. Those not given stimulating infections (non-infected-controls) did not show serum antibodies. Thus, such substances are not detectable, by the test used, within 6 days after a single infection. As expected from the earlier study (Hendricks, 1950), this test shows that the precipitin titer of the infected-controls stimulated with non-irradiated larvae varies with the numbers of stimulating infections (Table 2).

Irradiation, in addition to showing no interference with larval infectivity, also showed no interference with larval antigenicity, since antibodies in the usual concentrations were detected following infections. Antibodies reacting with adult antigen, and those reacting with larval antigen, both showed a relative increase with the number of stimulating infections (Table 2). The demonstration of such antibodies in mice harboring only "adult" worms is direct evidence against the view of certain workers that adult worms cause a local intestinal resistance with little or no production of circulating antibodies (Chandler, 1939, 1948).

The treatment of the larvae used in the stimulating infection, *i.e.*, irradiated or non-irradiated, did not affect the adult antibody titer, but the larval antibody, as would be expected, showed an over-all increase when the stimulating infections were with non-irradiated larvae. These results are interesting in view of the findings of Oliver-Gonzalez (1941). In his experiments on rabbits infected with normal (non-irradiated) larvae, the adult antibody was demonstrated by the formation of precipitates around living adult worms incubated in the serum. The precipitate was observed in serum collected 15–50 days after infection, and was again observed following superinfection when it was present in greatest quantity. The precipitin titer, using adult worm antigen, however, was negative throughout these infections. The success of demonstrating the adult antibody

by the precipitin test in the present study may be related to the preparation of the antigen and its use in greater concentration in the tests. The antigen used by Oliver-Gonzalez was an extraction of dried, pulverized adult worms in Cocoa's solution. This type of antigen is not as refined as that prepared by Melcher's (1943) technique used here, in which rapidly frozen worms are dried *in vacuo* (by the process of lyophilization) and treated to obtain the acid-soluble protein fraction. Moreover, the antigen used here was not diluted nearly as much as that in the tests conducted by Oliver-Gonzalez, thus would be more likely to detect a lower concentration of antibodies. If further study with this refined antigen shows that the adult antibody can be detected in human serum, the precipitin test as used here might be of great value in the early diagnosis of trichinosis.

It is interesting that the experimental mice stimulated with irradiated larvae, so that the infection was limited to the intestine, showed a rather high larval antibody titer (1:100), despite the fact that they theoretically received little or no larval stimulation. Of course, this could have resulted from antigenic stimulation by the infecting larvae, before the "adult" stage was reached. It is more likely, however, that the presence of these antibodies indicates that there is more than a single antigen present in this stage, as suggested for both stages by Oliver-Gonzalez (1941). Such common antigens must be closely related chemically, to react with antibodies produced against a single stage. As would be expected, there was a greater concentration of antibodies that react with adult antigen (1:293) than with larval antigen (1:100). This would seem to show that while both types of antibodies may arise from infections with irradiated larvae, the common antigen is less concentrated.

Comparison of the antibody titers of the experimentals and infected-controls gives further evidence for the presence of common antigens in the two stages of the parasite. The adult titers are similar in the two groups, but the larval titers are significantly higher in the infected-controls. If it can be assumed that the quantity of common antigen associated with the first two phases of the infections was similar in the two groups, then the increase in larval titer of the controls was due to the antigenic stimulation of the larvae deposited by the females. One might expect this increase to be even greater than shown, but it must be remembered that only the female worms, or about one-half of the total number of adults present, are contributing to this increase by larviposition.

Further research is needed to separate more completely these various antigens. It would seem advisable to produce infections limited to the musculature by inoculating larvae into the bloodstream. Comparison with the present infections (with "adults" only) should provide a basis for a more complete antigenic analysis.

It can be seen in the above discussion that stimulating infections with *T. spiralis* larvae, irradiated or non-irradiated, result in the production of acquired resistance in mice, as measured by the number of adult worms in the small intestine after a challenging infection. The precipitin test performed at the time of autopsy showed that the serum of these mice contained antibodies against *T. spiralis*. Analysis of the data shows there is a general relationship between the

number of worms recovered and the antibody titer. The challenging infection did not affect either of these factors. However, the increase in number of stimulating infections given prior to the challenging infection caused an over-all decrease in the number of worms and an increase in the precipitin titer. This relationship is not seen in the results of Series 1, in which the mice received only one stimulating infection. In this case there was not a reduction in worms despite the presence of a low antibody titer. This discrepancy in the over-all findings, noted in the earlier study (Hendricks, 1950), shows that antibodies are produced in sufficient quantity to be detected in this way as a result of one infection, but that the defensive mechanisms of the host are not yet effective in reducing the number of worms in the intestine.

The results of the present study strongly support the theory proposed by Oliver-Gonzalez (1940, 1941) that more than a single antibody is produced following experimental infections with *Trichinella spiralis*. The present study was not designed primarily to study the mechanism of this acquired resistance, so it is not the intent to imply that the antibodies detected are responsible for the resistance noted. Nevertheless, the present findings, when combined with those of Oliver-Gonzalez (*l.c.*), strongly suggest an antibody basis for this acquired resistance. The latter showed that adult worms and larvae incubated in serum from resistant animals show large masses of precipitates around the body openings. These precipitates are associated with immobilization, disintegration and, in many cases, death. If, as would seem likely, such action occurs in the bodies of resistant animals, it would explain the reduction in numbers of worms noted following reinfection. Moreover, because of the effect of such precipitates upon immobilization, it is likely that certain phagocytic cells could more easily attack the worms. This primary action of specific antibodies upon the worms, with secondary cellular cooperation, has been clearly demonstrated in rats resistant to the nematode, *Nippostrongylus muris* (Taliaferro and Sarles, 1939). Such *in vivo* studies for *T. spiralis* might reveal a similar mechanism. The findings of the present study and those of Oliver-Gonzalez open the way for this approach. The demonstration of the exact mechanism of resistance to *Trichinella spiralis* would be an important contribution to the knowledge of resistance to parasitic worms.

SUMMARY

This study of the dual antibody basis of resistance to *Trichinella spiralis* involved three groups of mice: the experimentals, given stimulating infections with irradiated larvae; the infected-controls, given the same infections with non-irradiated larvae; and the non-infected-controls which were not stimulated. By irradiation, infections in the experimental mice were limited to "adults" only, since larvae treated in this way fail to develop sexually. The degree of resistance acquired from the stimulating infections was measured by the number of adult worms recovered from the small intestine, following a challenging infection given to all three groups. The concentration of adult and larval antibodies at the time of autopsy was determined by the precipitin test. Comparisons were made, following 1–6 stimulating infections, of the degree of acquired resistance and the titer of serum antibodies.

Following one stimulating infection, the experimentals and the infected-controls showed about the same number of adult worms, but neither group developed acquired resistance, despite the presence of low adult and larval antibody titers.

Following 2–6 stimulating infections, while both groups showed acquired resistance, related, in general, to the increasing numbers of stimulating infections, that of the mice stimulated with irradiated larvae was less than that of those stimulated with non-irradiated larvae. This difference is discussed in light of an existing theory that *in utero* larvae are antigenically similar to the adults. Both groups showed an increase in adult and larval antibody titers related to the increasing numbers of stimulating infections. The adult antibody titer was about the same in the two groups but the larval antibody reached a higher titer in the infected-controls. The presence of larval antibodies in the experimental mice is offered as support for the suggestion of another worker, that such antibodies arise from antigens common to both stages of the parasite.

This work confirms and expands the information on the dual antibody basis of resistance to *T. spiralis*, and opens the way for further studies of the mechanism of this resistance.

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SEROLOGICAL CORRESPONDENCE AMONG THREE SPECIES OF PENAEID CRUSTACEA[1]

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Introduction

The morphological similarities of the three species of commercial shrimp, *Penaeus aztecus* Ives, *Penaeus setiferus* (Linnaeus), and *Penaeus duorarum* Burkenroad make the separation of them into their distinct systematic categories difficult. Immature specimens of the three species are indistinguishable. Burkenroad (1949) considers *P. aztecus* and *P. duorarum* to be more closely related to each other than either species is to *P. setiferus*. His conclusion is based on the observation that the two former species have a groove alongside the mid-dorsal carina of the sixth pleonic segment, while the latter species does not. *P. aztecus* and *P. duorarum* have, in addition, a reddish-brown, mid-lateral patch at the junction of the third and fourth pleonic segments. The patch is, however, greatly reduced in *P. aztecus*. There is no general agreement that these animals are three distinct species, or that *P. aztecus* and *P. duorarum* are closer to each other than either is to *P. setiferus*.

An analysis of the biochemical similarities and differences among organisms is possible through the use of serological comparisons of blood and other body proteins. In this paper are recorded the results of serological tests performed on the blood proteins of the above three species of penaeid Crustacea. We are unaware of any publications reporting intra-generic serological comparisons of species in the Genus *Penaeus* Latreille.

Materials and Methods

Antigens

The antigens compared in this study were obtained in the month of August, 1951, from Adams Creek off the Neuse River, North Carolina. Identification of the species from which the antigens were prepared was made by Mr. Carter Broad and other scientists from the North Carolina Institute of Fisheries Research, Morehead City, North Carolina.

Hemocyanins were obtained from the living shrimp by making an incision through the ventral hemocoel at the junction between the cephalothorax and the abdomen and allowing the wounded animals to flounder in 0.9 per cent. phosphate-buffered saline until dead. 'Merthiolate' in a final dilution of 1:5,000 was added as a bacteriostatic agent to the saline-hemocyanin mixture. The entire solution was filtered through Whatman filter paper No. 5 to clear it of any particulate matter. The filtrate was then bottled and stored in a refrigerator at 0° ± 2°C.

[1] These studies were aided by contract NR 163-012 with the Office of Naval Research, Department of the Navy.

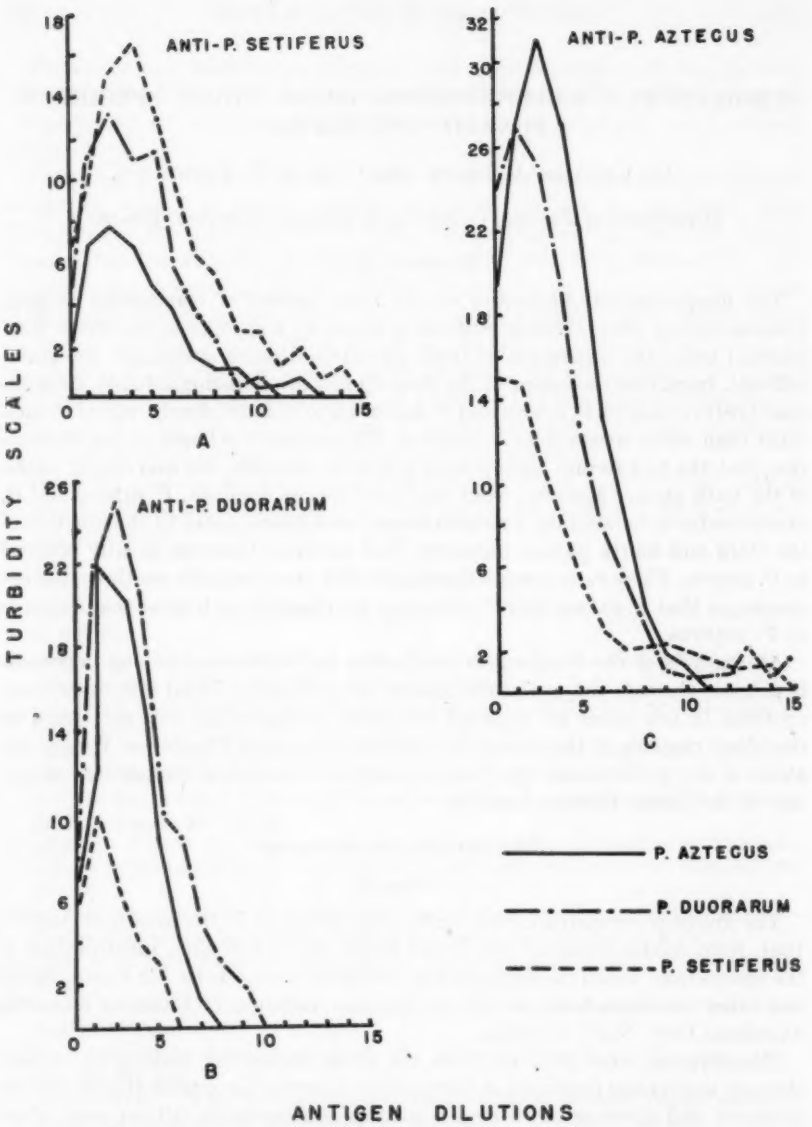

FIG. 1. Three series of precipitin titration curves showing the serological correspondence among three species of penaeid Crustacea. Homologous curves exceed all others. Turbidities are plotted along the ordinate; antigen-dilution tubes, ranging from 1:250 in tube No. 1, to 1:4,096,000 in tube No. 15, are plotted along the abscissa. Figure 1A shows the correspondence of *P. aztecus* and *P. duorarum* when reacted with the antiserum produced against *P. setiferus*. Figures 1B and 1C show the position of *P. setiferus* when reacted against anti-*P. duorarum* and anti-*P. aztecus* respectively; these figures also show the serological correspondence between *P. aztecus* and *P. duorarum*.

Serum protein concentrations of each saline-hemocyanin filtrate, as determined with the Leitz-Rouy Colorimeter, were: 0.87 grams per 100 c.c. of solution for *Penaeus aztecus;* 0.35 gms., for *P. setiferus;* and 0.70 gms., for *P. duorarum.* Since the concentration of protein in the initial preparation for *P. setiferus* was too low to enable suitable serological comparisons to be made, the entire solution was placed in a cellophane dialysis bag and evaporated before a running fan until a protein concentration of 0.75 gms. per 100 c.c. was obtained.

Antisera

All antisera were produced in normal, healthy, adult rabbits. Each rabbit received a pre-sensitizing injection of 0.25 c.c. of antigen given subcutaneously and then was permitted to rest for 30 days. Each rabbit then received two series of injections. A single series consisted of 0.5 c.c., 1.0 c.c., and 2.0 c.c., given intravenously on alternate days. Antisera which were obtained after the second series of injections contained enough precipitins to make possible the serological comparisons.

TABLE 1

Serological Relationships Among three species of Penaeus

ANTIGENS	ANTISERA		
	P. setiferus	*P. duorarum*	*P. aztecus**
P. setiferus	100	25	81
P. duorarum	73	100	92
P. aztecus	42	74	100

Numbers in the table represent percentage values which are calculated from the areas of the curves given in Figure 1. Homologous reactions are given a value of 100 per cent.

* The values for the antiserum to the proteins of *P. aztecus* represent only half-curve areas.

Trial bleedings of the rabbits were made eight days after the last injection of a series by taking whole blood from the median artery of the ear with a 22 gauge needle and a 10 c.c. syringe. Terminal bleedings were performed by direct cardiac puncture using 18 gauge needles and 50 c.c. syringes. The whole blood was placed in clean test tubes and allowed to clot, after which it was rimmed, then placed at 0° ± 2°C. for 12 to 18 hours; the serum containing the antibodies was then decanted. Antisera obtained by these methods were centrifuged, sterilized in Seitz filters, bottled in sterile vials, and stored at 0° ± 2°C. until used.

Method of Testing

Turbidities resulting from antigen-antibody reactions were measured with the Libby Photronreflectometer (1938), using testing methods outlined by Boyden and Defalco (1943), and Leone (1949). A series of doubling-dilutions of antigens from 1:250 to 1:4,096,000 was reacted with a constant amount of antiserum. In order to increase the specificity of the antisera so that heterologous reactions could more readily be distinguished from the homologous reactions, a dilution of

one part antiserum in two parts phosphate-buffered saline was employed in all tests (Boyden and Defalco, 1943).

Quantitative measurements were made of the precipitin turbidities which developed after 20 minutes in a dry air incubator maintained at 37°C.

All dilutions of the antigens were made with 0.9 per cent. phosphate-buffered saline with 'Merthiolate' added in a final dilution of 1:10,000 in order to prevent turbidities due to bacterial growth during the reaction time.

EXPERIMENTAL RESULTS

A summary of the serological tests is presented in Table 1. Plots of the turbidities are presented in Figure 1.

Table 1 shows that *P. aztecus* and *P. duorarum* are closely related to each other. The proteins of *P. duorarum*, moreover, react more strongly with the anti-*P. setiferus* serum than do the proteins of *P. aztecus*.

DISCUSSION

The serological data confirm the findings of Burkenroad on the systematic arrangement of the three species. The comparative biochemistry of the hemocyanins of the blood as determined by the serological methods described above could have two principal objections to it: (1) That the proteins, as prepared, are not representative of the animals, and (2) that the morphological similarities among the adults of the three species may have resulted in mixed lots of organisms from which the protein solutions were prepared.

With regard to (1), Leone (1949) has shown that hemocyanins are very stable under a great variety of preparatory procedures. Our treatment of the penaeid hemocyanin was relatively mild, and there probably was no significant effect on the species characteristics of these proteins. 'Merthiolate', in the concentrations used, has been shown by Leone (1949) not to alter the serological properties of hemocyanins.

Concerning (2), the identifications of the shrimp were made by professional marine biologists who are actively engaged in shellfisheries research. Our confidence in the serological data is based upon their practical skill in identifying these organisms. A greater accuracy in the field preparation of these animals into three groups is unlikely.

Hemocyanins, by their very nature, must be conservatively inherited (Leone, 1950). These proteins perform a multiplicity of functions, and are acceptable to all tissues of the organism. Substances such as these proteins, which are distributed throughout the entire body, provide a useful characteristic with which to make biochemical comparisons. That significant serological differences could be readily detected among the hemocyanins of these shrimp would support the separation of them into three distinct species.

SUMMARY

1. Serological comparisons of saline-hemocyanin filtrates of three species of shrimp, *Penaeus aztecus* Ives, *Penaeus setiferus* (Linnaeus), and *Penaeus duorarum* Burkenroad, were made, using a photoelectric turbidimeter.

2. The serological results place *P. aztecus* and *P. duorarum* closer to each other than either is to *P. setiferus*. This is in accord with Burkenroad's morphological arrangement of the three species.

3. *P. setiferus* is more similar to *P. duorarum* than it is to *P. aztecus*.

4. The serological differences are significant, and support the theory that these organisms are three distinct, but closely related, species.

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A NON-SELF-ADJOINT DIFFERENTIAL SYSTEM OF THE SECOND ORDER[1]

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We consider the differential system

$$(1) \qquad \begin{cases} y'(x, \lambda) = K(x, \lambda)z(x, \lambda), \\ z'(x, \lambda) = G(x, \lambda)y(x, \lambda), \end{cases}$$

$$(2') \qquad \begin{cases} \psi(a, \lambda) = \psi(b, \lambda), \\ \phi(a, \lambda) = \phi(b, \lambda), \end{cases}$$

where in (1) "prime" means differentiation with respect to x and in (2')

$$\psi(x, \lambda) = \gamma(x, \lambda)z(x, \lambda) - \delta(x, \lambda)y(x, \lambda),$$
$$\phi(x, \lambda) = \alpha(x, \lambda)z(x, \lambda) - \beta(x, \lambda)y(x, \lambda).$$

Carathéodory [3] has proved the existence of functions $y(x, \lambda)$ and $z(x, \lambda)$ which are absolutely continuous in x and continuous in the two variables (x, λ) on

$$XL: \begin{cases} X: a \leqq x \leqq b \\ L: L_1 < \lambda < L_2 \end{cases}$$

(λ may vary from $-\infty$ to $+\infty$) and which satisfy (1) almost everywhere on X, provided the following conditions hold:

i) $K(x, \lambda)$ and $G(x, \lambda)$ are defined on XL;

ii) for each fixed λ on L, $K(x, \lambda)$ and $G(x, \lambda)$ are measurable functions of x on X;

iii) for each fixed x on X, $K(x, \lambda)$ and $G(x, \lambda)$ are continuous functions of λ on L;

iv) there exists a Lebesgue summable function $M(x)$, such that on XL: $|K(x, \lambda)| < M(x)$ and $|G(x, \lambda)| < M(x)$.

In general, (1) and (2') are not compatible. There may exist values of λ for which they are compatible. Such values of λ are called *characteristic numbers* and the solutions corresponding to them are called *characteristic solutions*.

Investigations of many special cases of boundary conditions (2') have been made since the publication of the well-known Sturm-Liouville Memoirs.[2] Sturm [14] established fundamental properties of system (1) together with the boundary conditions

$$\begin{cases} \alpha_0 z(a, \lambda) - \beta_0 y(a, \lambda) = 0, \\ \gamma_0 z(b, \lambda) - \delta_0 y(b, \lambda) = 0, \end{cases}$$

where α_0, β_0, γ_0, and δ_0 are prescribed constants.

[1] Presented to the American Mathematical Society, April 21, 1950. Numbers in brackets refer to the bibliography at the end of the paper.

[2] See [7], [8], [9], [10], [11], [14], [15], and [16].

Picard [13] used the method of successive approximations to study the special system $K(x, \lambda) \equiv 1$, $G(x, \lambda) \equiv \lambda H(x)$, $H(x)$ of constant sign on X, $y(a) = y(b) = 0$.

Mason [12] and Bôcher [2] considered periodic boundary conditions. In particular, Mason used a normalizing method to prove the existence of an infinite system of characteristic numbers for system (1) with boundary conditions $y(a) = y(b)$, $z(a) = z(b)$, in the case where $K(x, \lambda) \equiv 1$ and $G(x, \lambda) \equiv \lambda A(x) - g(x)$. He also proved an oscillation theorem for this system. Bôcher, using different methods, removed the restriction on the form of $G(x, \lambda)$ but failed to establish uniqueness of the characteristic numbers, λ.

G. D. Birkhoff [1] extended the work of Mason and Bôcher to include the boundary conditions

$$\begin{cases} \alpha_0 z(a) - \beta_0 y(a) = \gamma_0 z(b) - \delta_0 y(b), \\ \alpha_1 z(a) - \beta_1 y(a) = \gamma_1 z(b) - \delta_1 y(b), \end{cases}$$

where α_i, β_i, γ_i, δ_i, $(i = 0, 1)$, are real and satisfy

$$\alpha_1\beta_0 - \alpha_0\beta_1 = \gamma_1\delta_0 - \gamma_0\delta_1 \neq 0.$$

Independently, Haupt [6] obtained results for the systems treated by Birkhoff through use of essentially different methods of attack.

Ettlinger [4], [5] extended the methods and results of Bôcher and Birkhoff [2; pp. 38–40] to the general, real, self-adjoint, linear system of the second order. He obtained both existence [4] and oscillation [5] theorems.

W. M. Whyburn [17] established existence and oscillation theorems for the non-self-adjoint systems (1)–(A) and (1)–(B), where

$$\text{(A)} \qquad \begin{cases} \alpha(\lambda)z(a, \lambda) - \beta(\lambda)y(a, \lambda) = 0, & \alpha \neq 0, \\ z(a, \lambda) = z(b, \lambda); \end{cases}$$

and

$$\text{(B)} \qquad \begin{cases} \alpha(\lambda)z(a, \lambda) - \beta(\lambda)y(a, \lambda) = 0, & \beta \neq 0, \\ y(a, \lambda) = y(b, \lambda). \end{cases}$$

He included these systems as special cases of differential systems with integral boundary conditions.

This paper is concerned with real solutions of the non-self-adjoint system (1)–(2), where

$$\text{(2)} \qquad \begin{aligned} &\text{(a)} \quad \psi(a, \lambda) = 0, \\ &\text{(b)} \quad \phi(a, \lambda) = \phi(b, \lambda). \end{aligned}$$

Hypotheses on the coefficients which are used from time to time are listed for reference:

i) $K(x, \lambda)$ and $G(x, \lambda)$ are defined on XL: $\begin{cases} X: a \leqq x \leqq b \\ L: L_1 < \lambda < L_2 ; \end{cases}$

ii) for each fixed λ on L, $K(x, \lambda)$ and $G(x, \lambda)$ are continuous functions of x on X;

iii) for each fixed x on X, $K(x, \lambda)$ and $G(x, \lambda)$ are continuous functions of λ on L;

iv) there exists an L-summable function $M(x)$, independent of λ, such that on XL: $|K(x, \lambda)| < M(x)$ and $|G(x, \lambda)| < M(x)$;

v) $K(x, \lambda) > 0$ and $-G(x, \lambda) > 0$ on XL^* where L^*: $L_1^* \leqq \lambda < L_2$ for some $L_1^* > L_1$;

vi) $K(x, \lambda)$ and $-G(x, \lambda)$ are non-decreasing functions of λ on L for each x on $a < x \leqq b$ and furthermore either $K(x, \lambda)$ or $-G(x, \lambda)$ is an actually increasing function of λ on L for a positive measured subset of any interval[3] $a < x \leqq x_1$;

vii) $y(a, \lambda) \equiv \gamma(a, \lambda)$ and $z(a, \lambda) \equiv \delta(a, \lambda)$ on L;

viii) $\gamma(a, \lambda)/\delta(a, \lambda)$ is a non-decreasing function of λ on L;

ix) $\delta(a, \lambda) \not\equiv 0$ on L;

x) $\alpha(x, \lambda)\delta(x, \lambda) - \beta(x, \lambda)\gamma(x, \lambda) \not\equiv 0$ on XL;

xi) $d/dx[\alpha(x, \lambda_n)/\beta(x, \lambda_n)] \leqq 0$ on X for each λ_n on L;

xii) $\alpha(b, \lambda)/\beta(b, \lambda)$ is a non-increasing function of λ on L.

The following notation is used:
for each λ on L let

$$
\begin{aligned}
m(K, \lambda) &\equiv \text{greatest lower bound of } K(x, \lambda) \text{ on } X,\\
m(-G, \lambda) &\equiv \text{g.l.b. } [-G(x, \lambda)] \text{ on } X,\\
M(K, \lambda) &\equiv \text{least upper bound of } K(x, \lambda) \text{ on } X,\\
M(-G, \lambda) &\equiv \text{l.u.b. } [-G(x, \lambda)] \text{ on } X,\\
m(\lambda) &= \text{minimum of } [m(K, \lambda), m(-G, \lambda)],\\
M(\lambda) &= \text{maximum of } [M(K, \lambda), M(-G, \lambda)].
\end{aligned}
$$

THE NON-SELF-ADJOINT SYSTEM

Consider the system

$$
\begin{cases} y' = Kz, \\ z' = Gy, \end{cases} \tag{1}
$$

$$
\begin{cases} \text{(a)} \quad \psi(a, \lambda) = 0, \\ \text{(b)} \quad \phi(a, \lambda) = \phi(b, \lambda), \end{cases} \tag{2}
$$

where

$$
\begin{aligned}
\psi(a, \lambda) &= \gamma(a, \lambda)z(a, \lambda) - \delta(a, \lambda)y(a, \lambda),\\
\phi(a, \lambda) &= \alpha(a, \lambda)z(a, \lambda) - \beta(a, \lambda)y(x, \lambda).
\end{aligned} \tag{3}
$$

Under hypothesis x), the system (1)–(2) is non-self-adjoint. A necessary and sufficient condition that the system be self-adjoint is that

$$
d_{24}K(b, \lambda) = d_{13}K(a, \lambda), \tag{4}
$$

[3] In case $K(x, \lambda)$ and/or $G(x, \lambda)$ are not suitably defined for $x > b$, let $K(x, \lambda) \equiv K(b, \lambda)$ and $G(x, \lambda) \equiv G(b, \lambda)$ for $x > b$.

where the d_{ij}'s are the six second order determinants formed from the matrix

$$\begin{pmatrix} -\delta(a,\lambda) & 0 & \gamma(a,\lambda)/K(a,\lambda) & 0 \\ -\beta(a,\lambda) & \beta(b,\lambda) & \alpha(a,\lambda)/K(a,\lambda) & -\alpha(b,\lambda)/K(b,\lambda) \end{pmatrix}.$$

For system (1)–(2), this yields

$$\begin{vmatrix} 0 & 0 \\ \beta(b,\lambda) & -\alpha(b,\lambda)/K(b,\lambda) \end{vmatrix} K(b,\lambda) = \begin{vmatrix} -\delta(a,\lambda) & \gamma(a,\lambda)/K(a,\lambda) \\ -\beta(a,\lambda) & \alpha(a,\lambda)/K(a,\lambda) \end{vmatrix} K(a,\lambda),$$

or

$$\alpha(a,\lambda)\delta(a,\lambda) - \beta(a,\lambda)\gamma(a,\lambda) = 0.$$

This contradicts hypothesis x).

Let $y(x,\lambda)$, $z(x,\lambda)$ be a solution of (1) which satisfies vii) and subject [18] system (1)–(2) to the polar coordinate type of transformation

$$\begin{aligned} y(x,\lambda) &= [U(x,\lambda)/\sqrt{\alpha^2(x,\lambda) + \beta^2(x,\lambda)}] \sin v(x,\lambda), \\ z(x,\lambda) &= [U(x,\lambda)/\sqrt{\alpha^2(x,\lambda) + \beta^2(x,\lambda)}] \cos v(x,\lambda), \end{aligned} \tag{5}$$

where

$$\begin{cases} U(a,\lambda) = [\alpha^2(a,\lambda) + \beta^2(a,\lambda)]^{\frac{1}{2}}[\gamma^2(a,\lambda) + \delta^2(a,\lambda)]^{\frac{1}{2}}, \\ v(a,\lambda) = \tan^{-1}[\gamma(a,\lambda)/\delta(a,\lambda)], \\ -\pi/2 < v(a,\lambda) \leqq \pi/2. \end{cases} \tag{5'}$$

It follows from hypothesis x) that

$$\alpha^2(x,\lambda) + \beta^2(x,\lambda) \neq 0$$

on XL and hence (5) is non-singular.

Upon differentiating (5) and eliminating y, y', z, z', one obtains

$$\begin{cases} (U/\sqrt{\alpha^2 + \beta^2})'/(U/\sqrt{\alpha^2 + \beta^2}) = (\tfrac{1}{2})(K + G)\sin 2v, \\ v' = K\cos^2 v - G\sin^2 v. \end{cases} \tag{6}$$

It is clear from the first equation of (6) that $U(x,\lambda)$ has exponential character. Hence any oscillatory properties which y and z have are due to $v(x,\lambda)$ alone.

Now let

$$\begin{cases} \sin\tau(x,\lambda) = \alpha(x,\lambda)/[\alpha^2(x,\lambda) + \beta^2(x,\lambda)]^{\frac{1}{2}}, \\ \cos\tau(x,\lambda) = \beta(x,\lambda)/[\alpha^2(x,\lambda) + \beta^2(x,\lambda)]^{\frac{1}{2}}, \end{cases} \tag{7}$$

where

$$\begin{cases} \tau(x,\lambda) = \tan^{-1}[\alpha(x,\lambda)/\beta(x,\lambda)], \\ -\pi/2 < \tau(x,\lambda) \leqq \pi/2. \end{cases} \tag{7'}$$

It follows from (3), (5), and (7) that

$$\phi(x,\lambda) = -U(x,\lambda)\sin[v(x,\lambda) - \tau(x,\lambda)]. \tag{8}$$

We introduce a notation f_c for $f(c, \lambda)$ to write equation 2(b) in the form

$$U_a \sin (v_a - \tau_a) = U_b \sin (v_b - \tau_b)$$

or

$$\sin (v_b - \tau_b)/[\sin (v_a - \tau_a) + \sin (v_b - \tau_b)] = U_a/[U_a + U_b]. \tag{9}$$

It follows from the exponential character of U that

$$0 < U_a/[U_a + U_b] < 1.$$

The existence of characteristic numbers for the system (1)–(2) is shown by first proving that there exist values of λ for which the left member of (9) is zero, and other λ values for which this is greater than unity. It then follows from the continuity of the left member that there are values of λ for which equation (9) holds, and hence system (1)–(2) is compatible.

THEOREM 1. On any closed sub-interval interval L' of L, the set S of all characteristic numbers of the system (1)–(2) is a closed pointset.

PROOF. S is the set of all zeros of $F(\lambda)$ on L', where

$$F(\lambda) = [U_a/(U_a + U_b)][\sin (v_a - \tau_a) + \sin (v_b - \tau_b)] - \sin (v_b - \tau_b).$$

When one recalls that $U_a/(U_a + U_b)$ is exponential in character and hence has no poles for finite λ, it is clear that $F(\lambda)$ is a continuous function of λ on L'. Upon making use of the fact that the set of zeros of a continuous function on a closed interval is closed, the theorem follows.

LEMMA 1. Under hypotheses i)–iv) and x), we have $0 < | v_a - \tau_a | < \pi$.

This follows from the definitions of v_a and τ_a as given in (5′) and (7′), when hypothesis x) is taken into account.

LEMMA 2. Under hypotheses i)–v), x) and xi), $[v(x, \lambda) - \tau(x, \lambda)]$ is an increasing function of x on X for each λ on L.

PROOF. If λ_n is a particular value of λ on L, it follows from (6) and hypothesis v) that

$$v'(x, \lambda_n) > 0$$

and this holds for each λ_n on L. From the definition of $\tau(x, \lambda)$ and hypotheses x) and xi), it follows that $\tau'(x, \lambda_n) \leqq 0$, and hence $[v(x, \lambda_n) - \tau(x, \lambda_n)]' > 0$.

LEMMA 3. Under hypotheses i)–vi), viii), and x), $v(x, \lambda)$ is an increasing function of λ on L for each x on X.

PROOF. From (6)

$$v'(x, \lambda) = K(x, \lambda) \cos^2 v(x, \lambda) - G(x, \lambda) \sin^2 (x, \lambda).$$

Let λ take a positive increment, find the increment of $v'(x, \lambda)$, and add and subtract

$$K(x, \lambda) \cos^2 v(x, \lambda + \Delta\lambda) + G(x, \lambda) \sin^2 v(x, \lambda + \Delta\lambda)$$

in the right member. Upon making use of the distributive law for differentiation and of well-known trigonometric identities, equation 6 becomes

$$(\Delta v)' = \Delta K \cos^2 v(x, \lambda + \Delta\lambda) - \Delta G \sin^2 v(x, \lambda + \Delta\lambda)$$
$$- (K + G) \sin [v(x, \lambda + \Delta\lambda) + v(x, \lambda)][(\sin \Delta v)/\Delta v]\Delta v$$

This is a first order differential equation in Δv and gives, on formal integration from a to x, the integral equation

$$\Delta v \exp\left[\int_a^x F(t, \lambda, \lambda + \Delta\lambda)\, dt\right]$$
$$= \int_a^x H(t, \lambda, \lambda + \Delta\lambda) \exp\left[\int_a^t F(s, \lambda, \lambda + \Delta\lambda)\, ds\right] dt + \Delta v(a, \lambda),$$

where

$$F(t, \lambda, \lambda + \Delta\lambda)$$
$$= [K(t, \lambda) + G(t, \lambda)] \sin [v(t, \lambda + \Delta\lambda) + v(t, \lambda)][\sin \Delta v(t, \lambda)]/\Delta v(t, \lambda),$$

and

$$H(t, \lambda, \lambda + \Delta\lambda) = \Delta K \cos^2 v(t, \lambda + \Delta\lambda) - \Delta G \sin^2 v(t, \lambda + \Delta\lambda).$$

From (5′)

$$\Delta v(a, \lambda) = v(a, \lambda + \Delta\lambda) - v(a, \lambda)$$
$$= \tan^{-1} [\gamma(a, \lambda + \Delta\lambda)/\delta(a, \lambda + \Delta\lambda)] - \tan^{-1} [\gamma(a, \lambda)/\delta(a, \lambda)].$$

Hence

$$\tan [\Delta v(a, \lambda)] = \frac{\gamma(a, \lambda + \Delta\lambda)/\delta(a, \lambda + \Delta\lambda) - \gamma(a, \lambda)/\delta(a, \lambda)}{1 + \gamma(a, \lambda + \Delta\lambda)\gamma(a, \lambda)/[\delta(a, \lambda + \Delta\lambda)\delta(a, \lambda)]}.$$

By hypothesis viii), the numerator here is positive or zero for all λ on L (since $\Delta\lambda > 0$). The sign of the denominator is likewise positive whenever

$$\gamma(a, \lambda)/\delta(a, \lambda) \geqq 0.$$

If

$$\gamma(a, \lambda)/\delta(a, \lambda) < 0,$$

then the continuity of γ and δ in λ assures that $\gamma(a, \lambda + \Delta\lambda)/\delta(a, \lambda + \Delta\lambda) \leqq 0$ for sufficiently small positive values of $\Delta\lambda$. Hence for sufficiently small $\Delta\lambda > 0$,

$$\tan [\Delta v(a, \lambda)] \geqq 0$$

for all λ on L. Since $v(a, \lambda)$ is a fourth or first quadrant angle and the tangent is an increasing function in these quadrants,

$$\Delta v(a, \lambda) \geqq 0.$$

By hypothesis vi)

$$H(x, \lambda, \lambda + \Delta\lambda) > 0,$$

and thus (by the positive character of exponential functions)

$$\Delta v(x, \lambda) > 0;$$

and this proves the lemma.

LEMMA 4. Under hypotheses i)–vi), viii), x), xii), $[v(b, \lambda) - \tau(b, \lambda)]$ is an increasing function of λ on L.

PROOF. This lemma follows directly from (7′) through use of hypothesis xii), Lemma 3, and the distributive law for increments.

LEMMA 5. Under hypothese; i)–vi), viii), and x)–xii), and for each non-negative integer n, the function

$$H(\lambda) = \sin(v_b - \tau_b)/[\sin(v_b - \tau_b) + \sin(v_a - \tau_a)]$$

takes on every value between zero and one at least once for values of λ between every pair of λ-values, λ_n and λ_{n+1}, where

$$v(b, \lambda_n) - \tau(b, \lambda_n) = n\pi - \pi/2,$$

and

$$v(b, \lambda_{n+1}) - \tau(b, \lambda_{n+1}) = n\pi + \pi/2.$$

In particular, then, the equation

$$H(\lambda) = \frac{U_a}{U_a + U_b}$$

is satisfied for at least one value of λ such that $\lambda_n < \lambda < \lambda_{n+1}$.

PROOF. When $v_b - \tau_b = n\pi$, $n = 0, 1, 2, \cdots$, it is clear that $H(\lambda) = 0$. By Lemma 4, if $v(b, \lambda_n) - \tau(b, \lambda_n) = n\pi - \pi/2$ and $v(b, \lambda_{n+1}) - \tau(b, \lambda_{n+1}) = n\pi + \pi/2$, then $\lambda_n < \lambda_{n+1}$. In proving that $H(\lambda)$ assumes values greater than unity for $\lambda_n < \lambda < \lambda_{n+1}$, four cases arise:

a) n even and $-\pi < v_a - \tau_a < 0$; then

$$H(\lambda_{n+1}) = 1/[1 + \sin\{v(a, \lambda_{n+1}) - \tau(a, \lambda_{n+1})\}] > 1;$$

b) n even and $0 < v_a - \tau_a < \pi$; then

$$H(\lambda_n) = 1/[1 - \sin\{v(a, \lambda_n) - \tau(a, \lambda_n)\}] > 1;$$

c) n odd and $-\pi < v_a - \tau_a < 0$; then

$$H(\lambda_n) = 1/[1 + \sin\{v(a, \lambda_n) - \tau(a, \lambda_n)\}] > 1;$$

d) n odd and $0 < v_a - \tau_a < \pi$; then

$$H(\lambda_{n+1}) = 1/[1 - \sin\{v(a, \lambda_{n+1}) - \tau(a, \lambda_{n+1})\}] > 1.$$

It follows from Lemma 1 that $H(\lambda)$ is defined for each λ on L with the exception of those λ's for which

$$\sin (v_a - \tau_a) = -\sin (v_b - \tau_b).$$

Since $\sin (v_a - \tau_a)$ and $\sin (v_b - \tau_b)$ are continuous functions of λ, $H(\lambda)$ is continuous with the exception of the poles indicated above.

In particular, since

$$0 < U_a/(U_a + U_b) < 1,$$

and since this function is continuous in λ on L, it follows that there exists at least one value of λ on $(\lambda_n, \lambda_{n+1})$ for which

$$H(\lambda) = U(a, \lambda)/[U(a, \lambda) + U(b, \lambda)].$$

LEMMA 6. Under hypotheses i)–v) and x), it follows that

$$-\pi + m(\lambda)(b - a) < v(b, \lambda) - \tau(b, \lambda) < \pi + M(\lambda)(b - a)$$

for all λ on L.

PROOF. From (6), we have

$$v'(x, \lambda) = K(x, \lambda) \cos^2 v(x, \lambda) - G(x, \lambda) \sin^2 v(x, \lambda).$$

Integration from a to b and subtraction of $\tau(b, \lambda)$ from both members yields

$$v(b, \lambda) - \tau(b, \lambda) = v(a, \lambda) - \tau(b, \lambda) + \int_a^b [K(t, \lambda) \cos^2 v(t, \lambda) - G(t, \lambda) \sin^2 v(t, \lambda)]\, dt. \tag{10}$$

Subtract and add $\int_a^b m(\lambda) \cos^2 v(t, \lambda)\, dt$ and $\int_a^b m(\lambda) \sin^2 v(t, \lambda)\, dt$ to the integral in the right member to get

$$\int_a^b \{[K(t, \lambda) - m(\lambda)] \cos^2 v(t, \lambda) + [-G(t, \lambda) - m(\lambda)] \sin^2 v(t, \lambda)\}\, dt + \int_a^b m(\lambda)\, dt.$$

From the definition of $m(\lambda)$, we have $K(t, \lambda) - m(\lambda) \geqq 0$ and $-G(t, \lambda) - m(\lambda) \geqq 0$. Hence

$$\int_a^b [K(t, \lambda) \cos^2 v(t, \lambda) - G(t, \lambda) \sin^2 v(t, \lambda)]\, dt \geqq m(\lambda) \int_a^b dt \geqq m(\lambda)(b - a).$$

Similarly, the definition of $M(\lambda)$ leads to

$$\int_a^b [K(t, \lambda) \cos^2 v(t, \lambda) - G(t, \lambda) \sin^2 v(t, \lambda)]\, dt \leqq M(\lambda) \int_a^b dt \leqq M(\lambda)(b - a).$$

These inequalities and (10) yield

$$m(\lambda)(b - a) \leqq [v(b, \lambda) - \tau(b, \lambda)] - [v(a, \lambda) - \tau(b, \lambda)] \leqq M(\lambda)(b - a). \tag{11}$$

Formulas (5′) and (7′) combine to yield

$$-\pi < v(a, \lambda) - \tau(b, \lambda) < \pi \tag{12}$$

on L. Combination of inequalities (11) and (12) gives

$$-\pi + m(\lambda)(b - a) < v(b, \lambda) - \tau(b, \lambda) < \pi + M(\lambda)(b - a). \tag{13}$$

DEFINITION. Let h be the smallest positive integer such that g.l.b. $M(\lambda)$ on L is less than $(h - \frac{3}{2})\pi/(b - a)$.

LEMMA 7. Under hypotheses i)–v) and x), the inequality

$$v(b, \lambda) - \tau(b, \lambda) < (h - \tfrac{1}{2})\pi$$

holds for at least one value of λ on L.

PROOF. By definition

$$\text{g.l.b. } M(\lambda) < (h - \tfrac{3}{2})\pi/(b - a).$$

Hence there exists at least one value, $\lambda = \lambda^*$, on L such that

$$M(\lambda^*) < (h - \tfrac{3}{2})\pi/(b - a).$$

By Lemma 6,

$$v(b, \lambda^*) - \tau(b, \lambda^*) < \pi + M(\lambda^*)(b - a) < (h - \tfrac{1}{2})\pi.$$

LEMMA 8. Under hypotheses i)–v) and x), if a positive integer j be chosen such that on L

$$\text{l.u.b. } m(\lambda) > (j + \tfrac{1}{2})\pi/(b - a),$$

then

$$v(b, \lambda) - \tau(b, \lambda) > (j - \tfrac{1}{2})\pi$$

for at least one λ on L.

PROOF. Since

$$\text{l.u.b. } m(\lambda) > (j + \tfrac{1}{2})\pi/(b - a),$$

there exists at least one value, $\lambda = \lambda^*$, on L such that

$$m(\lambda^*) > (j + \tfrac{1}{2})\pi/(b - a).$$

Hence, by Lemma 6,

$$v(b, \lambda^*) - \tau(b, \lambda^*) > -\pi + m(\lambda^*)(b - a) > (j - \tfrac{1}{2})\pi.$$

THEOREM 2. If j is finite and greater than h, then, under hypotheses i)–vi), viii) and x)–xii), there exist at least $(j - h)$ non-vacuous sets $S_1, S_2, \cdots, S_{j-h}$, of characteristic numbers for the system (1)–(2). Furthermore, if λ_n is a characteristic number belonging to S_n, then

$$(h + n - 1)\pi - \pi/2 < v(b, \lambda_n) - \tau(b, \lambda_n) < (h + n)\pi - \pi/2 \tag{14}$$

for $n = 1, 2, \cdots, (j - h)$.

PROOF. Start at $(h - \frac{1}{2})\pi$ on the real number axis and construct $(j - h)$ intervals of length π to obtain $I_n : [(h + n - 1)\pi - \pi/2, (h + n)\pi - \pi/2]$, $(n = 1, 2, \cdots, j - h)$. It follows from Lemmas, 5, 7, and 8 that there exists at least one characteristic number, λ_n (contained in S_n), which satisfies (14) for $n = 1, 2, \cdots, (j - h)$.

The possibility of additional sets of characteristic numbers arises from the fact that there exist values of λ for which

$$v(b, \lambda) - \tau(b, \lambda) \leqq h\pi - \pi/2,$$

and also values of λ for which

$$v(b, \lambda) - \tau(b, \lambda) \geqq j\pi - \pi/2.$$

Some of these λ-values may be characteristic numbers.

COROLLARY 2.1. If $m(\lambda)$ is such that j may be chosen arbitrarily large, then there exist infinitely many characteristic sets for the system (1)–(2).

THEOREM 3. Under hypotheses i)–vi), viii) and x)–xii), if λ_n is in S_n and λ_{n+1} is in S_{n+1}, then $\lambda_n < \lambda_{n+1}$.

PROOF. The theorem follows immediately from two applications of Theorem 2 and one of Lemma 4.

THEOREM 4. Under hypotheses i)–viii) and x)–xii), if λ_n belongs to S_n, then

$$\phi(x, \lambda_n) = U(x, \lambda_n) \sin [v(x, \lambda_n) - \tau(x, \lambda_n)]$$

vanishes on X at least $(h + n - 2)$ and at most $(h + n)$ times. In particular if

$$-\pi < v(a, \lambda_n) - \tau(a, \lambda_n) < 0,$$

then $\phi(x, \lambda_n)$ vanishes on X at least $(h + n - 2)$ and at most $(h + n)$ times; while if

$$0 < v(a, \lambda_n) - \tau(a, \lambda_n),$$

then $\phi(x, \lambda_n)$ vanishes on X at least $(h + n - 2)$ and at most $(h + n - 1)$ times.

PROOF. In view of the non-vanishing of $U(x, \lambda)$, it is sufficient for the proof to find the integral multiples of π that are assumed by $v(x, \lambda_n) - \tau(x, \lambda_n)$. Lemma 2 assures that this is an increasing function of x on X and hence takes its least value at $x = a$ and its greatest value at $x = b$. Lemma 1 gives one of the two cases

$$\text{(A)} \qquad -\pi < v(a, \lambda_n) - \tau(a, \lambda_n) < 0,$$

$$\text{(B)} \qquad 0 < v(a, \lambda_n) - \tau(a, \lambda_n) < \pi;$$

while Theorem 2 gives

$$(h + n - 1)\pi - \pi/2 < v(b, \lambda_n) - \tau(b, \lambda_n) < (h + n)\pi - \pi/2.$$

An actual count of the multiples of π taken on as x ranges from a to b completes the proof.

THEOREM 5. Under hypotheses i)–viii) and x)–xii), if λ_n belongs to S_n then $y(x, \lambda_n)$ vanishes on X at least $(h + n - 2)$ times and at most $(h + n)$. In par-

ticular, if

$$-\pi/2 < v(a, \lambda_n) \leqq 0,$$

then $y(x, \lambda_n)$ vanishes on X at least $(h + n - 1)$ and at most $(h + n)$ times; while if

$$0 < v(a, \lambda_n) \leqq \pi/2,$$

then $y(x, \lambda_n)$ vanishes on X at least $(h + n - 2)$ times and at most $(h + n - 1)$.

PROOF. It is sufficient here to find the integral multiples of π which $v(x, \lambda_n)$ may assume. The proof of Lemma 2 shows that $v(x, \lambda_n)$ is an actually increasing function of x on X and thus assumes its least value at $x = a$ and its greatest at $x = b$. An actual count after application of the inequalities in Theorem 2, formulas (7′), and in formulas (5′) completes the proof.

THEOREM 6. Under the hypotheses of Theorem 5, $z(x, \lambda_n)$ vanishes on X at least $(h + n - 2)$ times and at most $(h + n)$.

PROOF. Following the method of proof for Theorem 5, it is only necessary here to find the integral multiples of $\pi/2$ which $v(x, \lambda_n)$ may assume. By use of the inequality from Theorem 2 together with those from (5′) and (7′), an actual count may be made.

THEOREM 7. The sets S_j defined in Theorem 2 are closed.

This theorem follows from Theorem 1 and the continuity of $v(b, \lambda) - \tau(b, \lambda)$, along with an application of Theorem 2 and Lemma 4.

Any closed set is composed of an enumerable set and a perfect set. Let the sets S_j be decomposed as follows:

Let $S_j = \{s_{jk}\}$ where each s_{jk} is either a single point of S_j or else is a perfect subset of S_j. Furthermore, each s_{jk} is maximal in the sense that it is not a proper subset of any perfect subset of S_j.

THEOREM 8. Under hypotheses i)–viii) and x)–xii), if λ_j is an element of s_{jk} and λ_j^* is an element of s_{jk} different from λ_j and lying in a sufficiently small neighborhood of λ_j, then the zeros of the corresponding characteristic solutions of (1)–(2) separate each other.

PROOF. If s_{jk} consists of a single point, the theorem is vacuously satisfied. Suppose then that s_{jk} is a perfect set containing more than one point. Each point is thus a limit point of s_{jk} and in every neighborhood of λ_j there exist λ_j's distinct from λ_j.

Choose λ_j^* sufficiently close to λ_j, $\lambda_j^* \neq \lambda_j$, for $y(x, \lambda_j) = y(x)$ and $y(x, \lambda_j^*) = y^*(x)$ to have the same number of zeros on X. Continuity considerations make this choice possible. For definiteness, let $\lambda_j^* > \lambda_j$. Then from Lemma 3, there is at least one zero of $y^*(x)$ between each pair of zeros of $y(x)$. Since $y(x)$ and $y^*(x)$ have the same number of zeros on X, it follows that there can be only one zero of $y^*(x)$ between two consecutive zeros of $y(x)$. Since $\lambda_j^* > \lambda_j$, Lemma 3 ensures that the zeros of $y^*(x)$ and $y(x)$ do not coincide.

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ON THE SEPARATION OF THE SET OF PAIRS OF A SET*

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Among the basic problems in the foundation of mathematics there are many simply-conceived but apparently baffling situations. Such is the case when considering problems arising from the following question: If all of the pairs of elements of a given set M are divided into two mutually exclusive classes, under what conditions does M contain a subset, cardinally as large as M, all of whose pairs belong to the same one of these two classes? Obviously M must be infinite for the question to be of interest, and even then the number of elements in M has a direct bearing on the answer.

The Countable Case.[1] Suppose that (1) M is a countably infinite set, and (2) H and K are mutually exclusive collections such that $H + K = M^2$. Let N denote a subset of M such that either $N^2 \cdot H = 0$ or $N^2 \cdot K = 0$, and let G denote the collection of all such sets N. Is some element of G infinite? *Yes.*

Proof.[2] Suppose, on the contrary, that the answer were no. Let α denote a simple well-ordering of M and let a_0 denote the first element of α. For infinitely many elements a of α, $a_0 + a$ belongs to the same one of the collections H and K. Suppose then that α_1 is an infinite sequence of α such that if a is a term of α_1, $a_0 + a$ belongs to H. (Obviously a_0 does not belong to α_1.) It follows from the initial hypothesis that α_1 contains an element $\bar{a}$ such that for infinitely many different elements a of α_1, $\bar{a} + a$ belongs to K. Let a_1 denote the first such element $\bar{a}$ and let α_2 denote an infinite subsequence of α_1 such that if a is a term of α_2, $a_1 + a$ belongs to K. (As before, a_1 does not belong to α_2.) Let a_2 denote the first element of α_2 such that there exists an infinite subsequence α_3 of α_2 so that if a belongs to α_3, $a_2 + a$ belongs to H. This process may be continued so that there exist a sequence $a_0, a_1, a_2, \cdots$ of elements of α and a sequence $\alpha_1, \alpha_2, \alpha_3, \cdots$ of infinite subsequences of α such that (1) a_0 is the first element of α; (2) for each positive integer n, a_n belongs to α_n, and α_{n+1} is a subsequence of α_n; (3) if n is an *odd* positive integer and a is an element of α_n, then $a_{n-1} + a$ belongs to H; and (4) if n is an *even* positive integer and a is an element of α_n, then $a_{n-1} + a$ belongs to K. It follows that $a_0 + a_2 + a_4 + \cdots$ and $a_1 + a_3 + a_5 + \cdots$ are each infinite subsets of M and each belongs to G.

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[1] If M denotes a set, M^2 shall denote the collection of all subsets m of M such that m contains exactly *two* elements of M.

[2] Cf. Cornelis Visser, *On certain infinite sequences*, Proceedings of the Royal Academy of Science, Amsterdam, **40**, 1: 358–367, Theorem 4. 1937.

THE UNCOUNTABLE CASE. Let M be the set of real numbers, and let α denote a well-ordered sequence whose terms are the elements of M. Let H and K denote collections such that an element m of M^2 belongs to H if and only if the elements of m have the same order in α as in M; otherwise m belongs to K. Now let N be any uncountable subset of M. There exists a subsequence α' of α and an uncountable subset N' of N such that (1) α' is a well-ordering of N', and (2) no term in α' is preceded by uncountably many terms of α'. Since some point a of N' is a point of condensation of N' from both sides on M, a and some point of N' greater than a in M and in α' form a subset of M belonging to H, while a and some point of N' less than a in M but greater than a in α' form a subset of M belonging to K. Hence we may draw the following conclusion:

If M is a set such that $\aleph_1 \leqq |M| \leqq c$, there exists a separation of the elements of M^2 into two mutually exclusive sets H and K such that if N is a subset of M so that either $N^2 \cdot H = 0$ or $N^2 \cdot K = 0$, then N is countable.[3]

PARTIALLY ORDERED SETS. The application of the two preceding sections to partially ordered sets and lattices is evident. Pairs of elements in a partially ordered set may be divided into two classes depending upon whether the elements of a pair are "related" by the partial ordering or not. The following results may be obtained:

I. If a partially ordered set is countably infinite, then either it contains an infinite ordered subsystem or it contains an infinite subset no two elements of which are ordered.

II. There exists an uncountable partially ordered set M such that no uncountable subset is ordered and no uncountable subset is totally unordered.

OPEN QUESTION. Could the partially ordered set M in II have the following additional property: if a, b, and c are elements of M such that $a < b$ and $a < c$, then $b \lesseqgtr c$?

[3] An obvious approach to the problem of the continuum would be to prove that $|N|$ may be $\aleph_1$, if $\aleph_1 < |M|$.

ON SETS OF MATRICES HAVING A DELAYED COMMUTATIVITY PROPERTY[1]

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1. Introduction. In this paper we shall consider square matrices having elements in the complex field. Furthermore, these matrices have a delayed commutative property as defined and we show by the use of common invariant vectors that they may be reduced simultaneously by the same similarity transformation to matrices having triangular skeletons. By way of introduction we shall call attention to some known properties of commutative and quasi-commutative matrices and shall develop some lemmas and techniques to which we refer in the extensions and generalizations of these results.

We consider the finite set of $n \times n$ matrices $S = \{A, B, C, \cdots\}$ with characteristic roots $\alpha_1, \alpha_2, \cdots, \alpha_n$ of A, $\beta_1, \beta_2, \cdots, \beta_n$ of B, $\gamma_1, \gamma_2, \cdots, \gamma_n$ of C, $\cdots$, all notationally distinct but not necessarily otherwise possessed of dissimilarity. If the $\alpha_i, \beta_i, \gamma_i, \cdots$ $(i = 1, 2, \cdots, n)$ can be ordered such that for any rational function $f(x, y, z, \cdots)$ the characteristic roots of the matrix $f(A, B, C, \cdots)$ are given by $f(\alpha_i, \beta_i, \gamma_i, \cdots)$ $(i = 1, 2, \cdots, n)$ then the matrices of S are said to possess the "p" property.

A matrix A having elements a_{ij} such that $a_{ij} = 0$ for $i > j$ is said to be a triangular matrix or to have the triangular skeleton. We shall indicate a triangular matrix having diagonal elements a_{ii} $(i = 1, 2, \cdots, n)$ by the notation tri $\{a_{11}, a_{22}, \cdots, a_{nn}\}$. The same notation will be used for triangular block matrices. It is easy to show that if $S = \{A, B, C, \cdots\}$ is a set of triangular matrices then AB, A^I (if it exists) and $\lambda A + \mu B$ for scalars λ, μ are also triangular matrices. Furthermore, for any rational function $f(x, y, z, \cdots)$, the matrix $f(A, B, C, \cdots)$ is triangular and the diagonal elements of $f(A, B, C, \cdots)$ are $f(a_{ii}, b_{ii}, c_{ii}, \cdots)$ $(i = 1, 2, \cdots, n)$. It is clear that the diagonal elements of a triangular matrix are its characteristic roots. Obviously, then, if the matrices of S are triangular they possess the property "p".

It is well known that, for any $n \times n$ matrix A, there exists a unitary matrix U such that U^*AU has the triangular skeleton [1]. If there exists a matrix U such that U^*AU, U^*BU, U^*CU, $\cdots$ are all triangular matrices then we shall say that the matrices $A, B, C, \cdots$ have the *triangle property*. It is easy to show that a necessary and sufficient condition that U^*AU be triangular is that there exists a matrix P such that P^IAP is triangular. Since the characteristic roots of a matrix are invariant under a similarity transformation it follows immediately that any set of matrices having the triangle property also possesses the property "p".

[1] In part, this is an abridgment of a thesis submitted to the Faculty of the University of North Carolina in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the Department of Mathematics, 1948–49. The writer wishes to express his deep appreciation to Professor E. T. Browne for his direction of that work.

Commutative matrices, i.e., matrices satisfying the condition that

$$AB - BA = 0,$$

were studied extensively by Frobenius [2] and by many others. We shall use the standard notation $[A, B] = AB - BA$. McCoy [3] introduced quasi-commutative pairs of $n \times n$ matrices, for finite n, and proved that such matrices have the property "p". Roth introduced the idea of k-commutative matrices and showed by an illustration that k-commutative matrices do not necessarily have the "p" property [4, see illus. p. 2]. The writer proved that a general set of matrices, k-commutative in pairs, has the "p" property if the matrices of the set also have completely-triangular skeletons [5].

2. COMMUTATIVE MATRICES. For reference purposes we now establish, by the use of invariant vector spaces, some known properties of commutative matrices.

THEOREM 2.1. *If $R = \{A_1, A_2, \cdots, A_s\}$ is a finite set of $n \times n$ commutative matrices then the matrices of R have the triangle property.*

We first establish the following lemmas.

LEMMA 1. *Every pair of commutative matrices, A and B, always possess at least one common invariant vector.*

We show that for some characteristic root α of A and some characteristic root β of B there is an invariant vector η such that

$$A\eta = \alpha\eta \text{ and } B\eta = \beta\eta. \tag{1}$$

Let α be any characteristic root of A. If the nullity of $(A - \alpha I)$ is ν then there exists a ν-dimensional linear vector space Ξ of vectors ξ_i such that $A\xi_i = \alpha\xi_i$. Let $\xi_1, \xi_2, \cdots, \xi_\nu$ be a basis of this space. From the hypothesis, $[A, B] = 0$ and consequently

$$A(B\xi_i) = BA\xi_i = \alpha(B\xi_i) \qquad (i = 1, 2, \cdots, \nu). \tag{2}$$

It follows that the vector $B\xi_i$ is a vector of the linear space Ξ generated by $\xi_1, \xi_2, \cdots, \xi_\nu$. Then there exists ν^2 scalars k_{ij} such that

$$B\xi_i = \sum_{j=1}^{\nu} k_{ij}\xi_j \qquad (i = 1, 2, \cdots, \nu). \tag{3}$$

Let β be any characteristic root of the $\nu \times \nu$ matrix $K = (k_{ij})$. Then there exists a row vector $(x_1, x_2, \cdots, x_\nu)$ such that

$$\sum_{i=1}^{\nu} x_i k_{ij} = \beta x_j \qquad (j = 1, 2, \cdots, \nu). \tag{4}$$

It follows from (3) and (4) that the vector $\sum_{i=1}^{\nu} x_i\xi_i$ is the vector η of (1). That is, by using (3), then summing, we get

$$B\xi_i X_i = \sum_{j=1}^{\nu} k_{ij}\xi_j X_i \qquad (i = 1, 2, \cdots, \nu). \tag{5}$$

$$B\sum_{i=1}^{\nu} \xi_i X_i = \sum_{j=1}^{\nu} \left(\sum_{i=1}^{\nu} x_i k_{ij}\right)\xi_j = \beta \sum_{j=1}^{\nu} X_j \xi_j. \tag{6}$$

By writing $\sum_{i=1}^{\nu} \xi_i x_i = \eta$ we have the second equation of (1). However, η lies in the vector space and is thus an invariant vector of A with respect to the characteristic root α. Thus $A\eta = \alpha\eta$ and the lemma is established.

LEMMA 2. *Any finite set of $n \times n$ matrices $R = \{A_1, A_2, \cdots, A_s\}$, commutative in pairs, has at least one common invariant vector.*

We proceed by induction on r, the number of matrices in the set. By lemma 1, A_1 and A_2 have a common invariant vector space. Let the matrices $A_1, A_2, \cdots, A_{r-1}$ be any $(r-1)$ matrices of R which possess a common invariant vector space. Indicate a basis of this space by the vectors $\xi_1, \xi_2, \cdots, \xi_\nu$. Let A_r be any matrix of R except one of the set $A_1, A_2, \cdots, A_{r-1}$. Then $[A_j, A_r] = 0$ $(j = 1, 2, \cdots, r-1)$ and $A_j\xi_i = \alpha_j\xi_i$ $(j = 1, 2, \cdots, r-1;\ i = 1, 2, \cdots, \nu)$. Thus we have

$$(7) \quad A_j(A_r\xi_i) = A_r(A_j\xi_i) = \alpha_j(A_r\xi_i) \quad (j = 1, 2, \cdots, r-1;\ i = 1, 2, \cdots, \nu).$$

Hence, $(A_r\xi_i)$ is a vector of the space with basis $\xi_1, \xi_2, \cdots, \xi_\nu$. If we now replace B in (2) above by A_r and A in (2) by A_j and α by α_j, then (2) becomes (7). Thus (3), (4), (5) and (6) follow immediately from which we get the existence of a vector ζ which belongs to the space common to the matrices $A_1, A_2, \cdots, A_{r-1}$ and such that

$$(8) \qquad A_r\zeta = \alpha_r\zeta.$$

But, since ζ belongs to the space with basis $\xi_1, \xi_2, \cdots, \xi_\nu$, then

$$(9) \qquad A_j\zeta = \alpha_j\zeta \qquad (j = 1, 2, \cdots, r),$$

and lemma 2 is established.

LEMMA 3. *If the $n \times n$ triangular block matrices $A_i = \text{tri}\,\{\alpha_i, A_i^{(1)}\}$ $(i = 1, 2)$ are commutative then the $(n-1) \times (n-1)$ matrices $A_1^{(1)}$ and $A_2^{(1)}$ are commutative.* From the products, A_1A_2 and A_2A_1, in block fashion, this lemma is immediately evident.

We proceed directly to theorem 2.1 and use induction on n. If $n = 1$, then the matrices of $R = \{A_1, A_2, \cdots, A_s\}$ are already in the triangular skeleton. Our inductive hypothesis is the existence of a unitary matrix V_1 such that, for the $(n-1) \times (n-1)$ matrices $R = \{A_1^{(1)}, A_2^{(1)}, \cdots, A_s^{(1)}\}$

$$(10) \qquad V_1^*A_i^{(1)}V_1 = \text{tri}\,\{\alpha_1^{(i)}, \alpha_2^{(i)}, \cdots, \alpha_{n-1}^{(i)}\} \qquad (i = 1, 2, \cdots, s).$$

The A_i $(i = 1, 2, \cdots, s)$ have at least one common invariant vector. Let X be one of these column vectors which has been normalized. Build a unitary matrix W having X as first column. Then we have

$$(11) \qquad W^*A_iW = \text{tri}\,\{\alpha_i, A_i^{(1)}\} \qquad (i = 1, 2, \cdots, s).$$

By lemma 3, the $A_i^{(1)}$ $(i = 1, 2, \cdots, s)$ are commutative. By the inductive hypothesis there exists a unitary matrix $V = \text{tri}\,\{1, V_1\}$ such that, for $U = WV$ we have

$$(12) \qquad U^*A_iU = \text{tri}\,\{\alpha_i, T_i\} \qquad (i = 1, 2, \cdots, s).$$

Since T_i has the triangular skeleton the theorem is proved.

In what is probably his most referred to and perhaps also his best known paper Frobenius [2] proved the following theorem.

THEOREM 2.2. *Let* $R = \{A_1, A_2, \cdots, A_s\}$ *be a finite set of* $n \times n$ *matrices, commutative in pairs and having elements in a field* F. *Then the matrices of* R *have the "p" property.*

This theorem is a direct consequence of theorem 2.1.

3. QUASI-COMMUTATIVE MATRICES. By definition, two $n \times n$ matrices A and B are quasi-commutative if $[A, B] = C$ where C commutes with both A and B. By the use of invariant vectors we shall establish the following theorem, due to McCoy [3].

THEOREM 3.1. *If* A *and* B *are quasi-commutative matrices, where* $[A, B] = C$, *then* C *is nilpotent.*

For, let γ be any characteristic root of C and let $X_1, \cdots, X_\nu$ be a basis of an invariant vector space of C with respect to γ. Then

$$CX_i = \gamma X_i \qquad (i = 1, 2, \cdots, \nu). \tag{13}$$

Since $[A, C] = 0$ and $[B, C] = 0$ we have AX_i and BX_i also belonging to this vector space. Thus there exists ν^2 scalars k_{ij} and ν^2 scalars l_{jt} such that

$$AX_i = \sum_{j=1}^{\nu} k_{ij} X_j, \qquad BX_i = \sum_{j=1}^{\nu} l_{ij} X_j \qquad (i = 1, 2, \cdots, \nu). \tag{14}$$

From (14) it follows that

$$BAX_i = \sum_{j=1}^{\nu} k_{ij} BX_j = \sum_{j=1}^{\nu} k_{ij} \sum_{t=1}^{\nu} l_{jt} X_t \qquad \text{and that} \tag{15}$$

$$ABX_i = \sum_{j=1}^{\nu} l_{ij} AX_j = \sum_{j=1}^{\nu} l_{ij} \sum_{t=1}^{\nu} k_{jt} X_t. \tag{16}$$

Hence, from (13), (15) and (16)

$$\gamma X_i = (AB - BA)X_i = \Big(\sum_j \sum_t l_{ij} k_{jt} - \sum_j \sum_t k_{ij} l_{jt}\Big) X_t \qquad (i = 1, 2, \cdots, \nu). \tag{17}$$

Now, (17) is true for $i = 1, 2, \cdots, \nu$. The vectors $X_1, X_2, \cdots, X_\nu$ are linearly independent and thus it follows that the matrices K and L must satisfy the condition that $[L, K] = \gamma I$. From this it follows that $\gamma = 0$. Since γ was any characteristic root of C then C is nilpotent.

Since $[L, K] = 0$ then, by lemma 1, K and L possess a common invariant vector $U = (u_1, u_2, \cdots, u_\nu)$ such that

$$KU = \alpha U \text{ and } LU = \beta U \tag{18}$$

for some characteristic roots α of K and β of L. From (14) we have

$$AX_i u_i = \sum_{j=1}^{\nu} k_{ij} X_j u_i \qquad (i = 1, 2, \cdots, \nu) \tag{19}$$

and thus, from (18) and (19)

$$A\sum_{i=1}^{p} u_i X_i = \sum_{j=1}^{p}\left(\sum_{i=1}^{p} k_{ij} u_i\right) X_j = \alpha \sum_{j=1}^{p} u_j X_j .$$

Hence it is seen that α is a characteristic root of A and that the vector $\eta = \sum_{i=1}^{p} u_i X_i$ is an invariant vector of A with respect to this charcteristic root α. By a similar argument β is a characteristic root of B and η is an invariant vector of B with respect to the characteristic root β of B. Thus we have established the following

LEMMA 5. *If A and B are $n \times n$ quasi-commutative matrices then A and B possess a common invariant vector space.*

We shall now establish

THEOREM 3.2. *If A and B are $n \times n$ quasi-commutative matrices then they have the triangle property.*

By lemma 5, A and B possess a common invariant vector X, such that $AX = \alpha X$ and $BX = \beta X$. There exists a unitary matrix P, having the elements of its first column proportional to the elements of X, such that $P^*AP = \text{tri}\ \{\alpha, A_1\}$, $P^*BP = \text{tri}\ \{\beta, B_1\}$. The matrices A_1 and B_1 are easily shown to be quasi-commutative matrices. The theorem is immediately obvious if A and B are 1×1 matrices. The inductive hypothesis that for $(n-1) \times (n-1)$ quasi-commutative matrices A_1 and B_1 there exists an $(n-1) \times (n-1)$ unitary matrix Q such that Q^*A_1Q and Q^*B_1Q are both triangular, gives immediately, upon setting $U = PM$, where $M = \text{tri}\ \{1, Q\}$,

$$U^*AU = \text{tri}\ \{\alpha_1, \alpha_2, \cdots, \alpha_n\}, \qquad U^*BU = \text{tri}\ \{\beta_1, \beta_2, \cdots, \beta_n\}$$

and the theorem is proved.

We have as an immediate consequence of theorem 3.2, the following corollary:

COROLLARY. *If A and B are $n \times n$ quasi-commutative matrices they possess the "p" property.*

It should be noted that theorem 2.1 is a special case of theorem 3.2.

4. SEMI-QUASI-COMMUTATIVE MATRICES. We now give a one sided extension of quasi-commutative matrices.

DEFINITION. If the $n \times n$ matrices A and B have the delayed commutativity property satisfying the condition that $[A, B] = C$, $[C, B] = 0$, $[C, A] = D$, $[D, A] = 0$ then we shall define A and B to be semi-quasi-commutative matrices.

It is easy to show that D commutes with B and with C and thus the chain is finite. It is clear that A and C have the triangle property and that D is nilpotent. Also C is nilpotent. For,

$$C^r = C^{r-1}[A, B] = [(C^{r-1}A), B] \qquad (r = 1, 2, \cdots).$$

Thus, C^r $(r = 1, 2, \cdots)$ has trace zero. Now, C either possesses characteristic roots each having the value zero or there are some nonzero characteristic roots

$\gamma_1, \gamma_2, \cdots, \gamma_t$ having multiplicities $\mu_1, \mu_2, \cdots, \mu_t$ respectively. In this case the linear system $\sum_{i=1}^{t} \mu_i \gamma_i^r = 0$ $(r = 1, 2, \cdots, t)$ is satisfied if and only if

$$\begin{pmatrix} 1 & 1 & \cdots & 1 \\ \gamma_1 & \gamma_2 & \cdots & \gamma_t \\ \cdot & \cdot & \cdot & \cdot \\ \gamma_1^{t-1} & \gamma_2^{t-1} & \cdots & \gamma_t^{t-1} \end{pmatrix} \begin{pmatrix} \gamma_1 \\ \gamma_2 \\ \cdot \\ \gamma_t \end{pmatrix} = 0,$$

which leads to a contradiction of the hypothesis that $\gamma_i \neq 0$ $(i = 1, 2, \cdots, t)$. Thus we have proved

THEOREM 4.1. *Semi-quasi-commutative matrices have nilpotent commutes.*

We next show that semi-quasi-commutative matrices have the triangle property. This is done by showing that they possess a common invariant vector space, and, by using induction on n, the order of the matrices.

The following lemmas are first established.

LEMMA 6. *If ξ is any common invariant vector of the two $n \times n$ matrices A and B with respect to the respective characteristic roots α and β then ξ is an invariant vector of C, where C is $[A, B]$.*

This is immediately evident since $C\xi = (\alpha\beta - \beta\alpha)\xi = 0$. As a direct consequence of lemma 6, we have

LEMMA 7. *If A and B have a common invariant vector ξ then ξ is an invariant vector of A, B, and all commutes associated with this pair.*

LEMMA 8. *Let A, B, C be $n \times n$ matrices such that $[A, B] = C$ and such that $\xi_1, \xi_2, \cdots, \xi_s$, $s \geqq 1$ is a basis of a common invariant vector space of A and C. If $A(B\xi_i) = \alpha(B\xi_i)$ and $C(B\xi_i) = \gamma(B\xi_i)$ $(i = 1, 2, \cdots, s)$ then there exists a common invariant vector space of A, B and C.*

Since $(B\xi_i)$ $(i = 1, 2, \cdots, s)$ belongs to the space $\xi_1, \xi_2, \cdots, \xi_s$, this lemma follows in exactly the same way as lemma 1.

By applying lemma 8 to the semi-quasi-commutative matrices A and B, we find that A, B and C have a common invariant vector. By lemma 7 there exists a common invariant vector of the matrices A and B and of all the associated commutes, in this case C and D. As in (11), there exists a unitary matrix P_1 such that $P_1^* A P_1 = \text{tri}\,\{\alpha, A^{(1)}\}$, $P_1^* B P_1 = \text{tri}\,\{\beta, B^{(1)}\}$ and $P_1^* C P_1 = \text{tri}\,\{0, C^{(1)}\}$. By an argument similar to that of lemma 3, $A^{(1)}$, $B^{(1)}$ and $C^{(1)}$ are semi-quasi-commutative. Continuing by induction there exists a unitary matrix P such that for A, B and C we have

$$P^*AP = \text{tri}\,\{\alpha_1, \alpha_2, \cdots, \alpha_n\}, \qquad P^*BP = \text{tri}\,\{\beta_1, \beta_2, \cdots, \beta_n\},$$

$$P^*CP = \text{tri}\,\{0, \cdots, 0\}.$$

This completes the proof of the theorem below:

THEOREM 4.1. *Semi-quasi-commutative matrices have the triangle property.*

The following corollary is a direct consequence of this theorem:

COROLLARY. *Semi-quasi-commutative matrices have the "p" property.*

5. QUASI-k,l-COMMUTATIVE MATRICES. We now consider quasi-k,l-commutative matrices and show that quasi-2,2-commutative matrices possess the triangle property and, consequently the "p" property.

DEFINITION. If two $n \times n$ matrices, A and B, have the delayed commutativity property that $[A, B] = C$, $[C, A] = Q$, $[C, B] = R$, where both Q and R commute with each of A, B then we shall define A and B to be quasi-2,2-commutative.

In proving lemma 2 we actually proved a more general lemma which we state for future reference.

LEMMA 9. *Let the $n \times n$ matrices A_i $(i = 1, 2, \cdots, s-1)$ have a common invariant vector space corresponding to the characteristic roots α_i of A_i $(i = 1, 2, \cdots, s-1)$ and let ξ_i $(i = 1, 2, \cdots, t)$, $t \geqq 1$ denote a basis of this space. If A_s is any matrix such that the vectors $A_s\xi_i$ $(i = 1, 2, \cdots, t)$ belong to this space then there exists a common invariant vector space of the matrices A_i $(i = 1, 2, \cdots, s)$ corresponding to the characteristic roots α_i $(i = 1, 2, \cdots, s)$.*

Let A and B be quasi-2,2-commutative as defined. Then A and C are quasi-commutative and by lemmas 5 and 7, the matrices A, C and Q have a common invariant vector space. Since R commutes with both A and C we have, by lemma 9, that A, C, Q and R have a common invariant vector space. Also, since Q and R are primary commutes of the quasi-commutative pairs A, C and B, C respectively, Q and R are nilpotent.

Now, let γ be a characteristic root of C with respect to the vector space $\xi_1, \xi_2, \cdots, \xi_\nu$ common to A, C, Q and R. Then, as in (9) $C\xi_i = \gamma\xi_i$ $(i = 1, 2, \cdots, \nu)$ and, since $[C, B] = R$, $B\xi_i$ belongs to this space. Proceeding in exactly the same way as in the case of quasi-commutative matrices we see that C is nilpotent and that A and B have a common invariant vector. By lemma 7, A, B, C, Q and R have a common invariant vector. Then, as in the case for commutative matrices and quasi-commutative matrices, there exists a unitary matrix P such that P^IAP, P^IBP, P^ICP, P^IQP, and P^IRP are all triangular matrices. Thus we have

THEOREM 5.1. *A pair of quasi-2,2-commutative matrices has the triangle property.*

COROLLARY. *Quasi-2,2-commutative matrices have the "p" property.*

There are several commute patterns somewhat like the quasi-k,l-commutative matrices. For example, consider the $n \times n$ matrices A and B such that $[A, B] = C$ is nonderogatory and form the single chains of commutes

$$A_s = \sum_{i=0}^{s} (-1)^i \binom{s}{i} C^{s-i} A C^i \qquad \text{and} \qquad B_t = \sum_{i=0}^{t} (-1)^i \binom{t}{i} C^{t-i} B C^i.$$

Consider also, the $n \times n$ matrices A and B with the postponed commutativity satisfying the conditions that $[A, B] = C$, $[C, B] = Q$, $[Q, C] = X$, $[Q, B] = Y$ where X and Y each commutes with A and B. Then by theorem 5.1, lemma 8, and for nonsingular B, it is easy to show that A and B have the triangle property.

It should be noted that it is not necessary that two associated chains be of the same length. The following illustrates this point, and, although these matrices

have a striking similarity to both the semi-quasi-commutative and the quasi-2,2-commutative matrices, they are neither. Let

$$A = \begin{pmatrix} -7 & -4 & 5 \\ -15 & -19 & 11 \\ -22 & -13 & 16 \end{pmatrix}, \quad B = \begin{pmatrix} -2 & -3 & 3 \\ -2 & 2 & 0 \\ -7 & -4 & 6 \end{pmatrix}, \quad P = \begin{pmatrix} 8 & 4 & -5 \\ -5 & -2 & 3 \\ -1 & -1 & 1 \end{pmatrix}.$$

Then $[A, B] = C \neq 0$, $[C, A] = 0$, $[C, B] = D \neq 0$, $[D, C] = 0$ and $[D, B] \neq 0$. P^IAP and P^IBP are both of like triangular pattern.

It is not sufficient to require only the first commute to be nilpotent. For, if the 3×3 matrices $A = (a_{ij})$ and $B = (b_{ij})$ are given by $a_{11} = a_{22} = b_{13} = b_{22} = 2$, $a_{33} = b_{11} = 3$, $a_{12} = b_{21} = b_{23} = b_{31} = b_{32} = 1$, $a_{13} = a_{21} = a_{23} = a_{31} = a_{32} = b_{12} = b_{33} = 0$ then $[A, B]^3 = 0$ but $[[A, B], A]$ has rank 3.

6. SETS OF QUASI-COMMUTATIVE MATRICES. We now extend the idea of quasi-commutative pairs of matrices to quasi-commutative sets of matrices. This generalizes the concept of quasi-commutative matrices.

DEFINITION. If the $n \times n$ matrices of the set $S = \{A_1, A_2, \cdots, A_r\}$ are such that each $[A_i, A_j]$ commutes with each A_t $(i, j, t = 1, 2, \cdots, r; i < j)$ then we define the matrices of S to be quasi-commutative in the set.

Since A_i, A_j are quasi-commutative then $[A_i, A_j] = B_{ij}$ is nilpotent and also the B_{ij} $(i, j = 1, 2, \cdots, r)$ are commutative in pairs. A_1 and A_2 of S are quasi-commutative and it follows from lemma 7 that there exists an invariant vector space, Γ, common to A_1, A_2, B_{12} with respect to the characteristic roots $\alpha_1^{(1)}$, $\alpha_1^{(2)}$ and zero respectively. Let a basis of the space, Γ, be $X_1, X_2, \cdots, X_\lambda$, $\lambda \geqq 1$. Then

$$A_jX_i = \alpha_1^{(j)}X_i, \qquad B_{12}X_i = 0, \qquad (i = 1, 2, \cdots, \lambda; j = 1, 2).$$

However, B_{13} commutes with each of A_1, A_2, B_{12} and it follows that

$$A_j(B_{12}X_\mu) = \alpha_1^{(j)}(B_{13}X_\mu), \qquad B_{12}(B_{12}X_\mu) = 0 \quad (\mu = 1, 2, \cdots, \lambda; j = 1, 2).$$

Therefore, $(B_{13}X_\mu)$ $(\mu = 1, 2, \cdots, \lambda)$ is a vector of the invariant space, Γ, common to A_1, A_2, B_{12} and, by lemma 9, there is a common invariant vector of A_1, A_2, B_{12}, B_{13}. We proceed by induction, to show that the matrices $A_1, A_2, B_{12}, B_{13}, \cdots, B_{r-1,r}$ have a common invariant vector. Suppose the matrices $S_1 = \{A_1, A_2, B_{12}, B_{13}, \cdots, B_{kl}\}$ have a common invariant vector space with a basis $Y_1, Y_2, \cdots, Y_\nu$, $\nu \geqq 1$. Let B_{ij} be any first commute not in S_1. Since B_{ij} commutes with each matrix of S_1, we have

$$A_1(B_{ij}Y_\mu) = \alpha_1^{(1)}(B_{ij}Y_\mu), \qquad A_2(B_{ij}Y_\mu) = \alpha_1^{(2)}(B_{ij}Y_\mu) \text{ and } B_{\theta\phi}(B_{ij}Y_\mu) = 0$$
$$(\mu = 1, 2, \cdots, \nu; (\theta, \phi) = (1, 2), \cdots, (k, l)).$$

Thus, $(B_{ij}Y_\mu)$ $(\mu = 1, 2, \cdots, \nu)$ belongs to the common invariant vector space and there exists, by lemma 9, a common invariant vector of the set $S_2 = \{S_1; B_{ij}\}$ with respect to the characteristic roots $\alpha_1^{(1)}, \alpha_1^{(2)}, 0, 0, \cdots, 0$.

We shall now show, again by induction, that the set of matrices $A_1, A_2, \cdots,$

$A_r, B_{12}, \cdots, B_{r-1,r}$ has a common invariant vector with respect to the characteristic roots $\alpha_1^{(1)}$ of A_1, $\alpha_1^{(2)}$ of $A_2, \cdots, \alpha_1^{(r)}$ of A_r, 0 of $B_{12}, \cdots$, and 0 of $B_{r-1,r}$.

We shall assume that there is a common invariant vector space of the matrices $A_1, A_2, \cdots, A_l, B_{12}, \cdots, B_{r-1,r}$, $l < r$, and let a basis of this space be $Z_1, Z_2, \cdots, Z_\rho$, $\rho \geqq 1$. Since

$$A_{l+1}B_{ij} = B_{ij}A_{l+1} \qquad ((i, j) = (1, 2), \cdots, (r-1, r))$$

it follows that

$$B_{ij}(A_{l+1}Z_\mu) = 0 \quad ((i, j) = (1, 2), \cdots, (r-1, r)), \qquad (\mu = 1, 2, \cdots, \rho)$$

and, since

$$A_iA_{l+1} - A_{l+1}A_i = B_{i,l+1} \qquad (i = 1, 2, \cdots, l),$$

it follows that

$$A_i(A_{l+1}Z_\mu) = \alpha_1^{(i)}(A_{l+1}Z_\mu) \qquad (i = 1, 2, \cdots, l;\ \mu = 1, 2, \cdots, \rho).$$

Consequently, by lemma 9, there exists a common invariant vector of the matrices of the set $S_2 = \{A_1, A_2, \cdots, A_l, A_{l+1}, B_r, \cdots, B_{r-1,r}\}$, corresponding to the characteristic roots $\alpha_1^{(1)}, \alpha_1^{(2)}, \cdots, \alpha_1^{(l)}, \alpha_1^{(l+1)}, 0, \cdots, 0$ respectively. Thus we have established the existence of a common invariant vector of the matrices $A_1, A_2, \cdots, A_r, B_{12}, \cdots, B_{r-1,r}$ corresponding to the characteristic roots $\alpha_1^{(1)}, \alpha_1^{(2)}, \cdots, \alpha_1^{(r)}, 0, \cdots, 0$ respectively.

There exists then a unitary matrix P such that $P^*A_iP = \text{tri}\ \{\alpha_1^{(1)}, A_i^{(1)}\}$ and $P^*B_{ij}P = \text{tri}\ \{0, B_{ij}^{(1)}\}$. It is easy to show that there exists a unitary matrix U such that

$$U^*A_iU = \text{tri}\ \{\alpha_1^{(i)}, \alpha_2^{(i)}, \cdots, \alpha_n^{(i)}\} \qquad \text{and} \qquad U^*B_{ij}U = \text{tri}\ \{0, 0, \cdots, 0\}$$
$$(i, j = 1, 2, \cdots, r).$$

This establishes, for the matrices $A_1, A_2, \cdots, A_r$, the triangle property from which follows the "p" property and we have

THEOREM 6.1. *If the matrices $A_1, A_2, \cdots, A_r$ are quasi-commutative in the set then they are reducible simultaneously, by the same similarity transformation, to matrices having the triangular skeleton.*[2]

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[2] This theorem appeared in the writer's dissertation. It has since appeared in a paper by Drazin, Dungey and Gruenberg [6]. Their paper is based on a paper by McCoy [7].

NATURAL HYBRIDS IN THE GENUS SARRACENIA

I. History, Distribution and Taxonomy

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Plates 1 to 7

Introduction

During the decade from 1880 to 1890, when *Sarracenia* hybrids were popular horticultural plants in England, there were three fairly complete though somewhat conflicting treatments of these plants from a horticultural viewpoint by Masters (1881), "S. G."[1] (1885), and Nicholson (1887). Three subsequent papers by Macfarlane (1908), Harper (1918), and Bell (1949) have dealt with the genus *Sarracenia* as a natural unit of the native flora. The main concern of these latter papers was the morphology, distribution, and taxonomy of the species themselves; and although Macfarlane (1908) does list most of the horticultural hybrids, the natural hybrids receive rather short treatment, especially as to descriptions, in all of these last three works on the genus. As a result, proper identification of these not too uncommon hybrid elements of the southeastern flora has not been made. Thus, of twenty sheets of material in the U. S. National Herbarium labeled or annotated *Sarracenia Catesbaei*, only three and a half sheets were actually of these *S. flava* × *S. purpurea* hybrids. The rest are referable as follows: three sheets of *S. flava*, eight sheets of *S. Sledgei*, one-half sheet of *S. purpurea*, two sheets of *S. rubra* f. *Jonesii*, one sheet of *S. rubra*, and two sheets of the hybrid *S. Drummondii* × *S. flava*! Part of this confusion is probably due to the varied history of the name *S. Catesbaei* (which will be reviewed later). Part is undoubtedly due to the brief horticultural popularity of these hybrids and the resultant ambiguity in the application of Latin names to these plants by horticulturists. The latter seemingly desired a stabilized nomenclature, but seldom followed the few rules of description, synonomy, and priority necessary to achieve this goal. However, the largest part of the taxonomic confusion in regard to natural *Sarracenia* hybrids, as well as natural hybrids in general, is due to the taxonomists themselves. These individuals, finding natural hybrids an interesting but extremely minor segment of any given flora, have justifiably devoted their time and energies to larger problems and/or ones more promising of solution with the material available. This has resulted in the continued postponement of action on rules and methods of dealing with hybrids taxonomically until we are

[1] An article on *New Hybrid Sarracenias* in *The Garden* **28**: 217–219, 1885, was signed only "S. G." These were not the editor's initials and thus far all attempts to trace the identity of "S. G." have failed. The publishers of this now defunct journal very kindly searched their records in an effort to find the name of the author, but also without success. Since the rest of the citation is complete, however, the initials *per se* are used as a reference here and farther on as an authority for a horticultural name.

now faced with an acute need for such rules and do not have them. The urgency of the situation was realized by the members of the 1950 International Botanical Congress. Action was taken there which may result in the desired rules, provided taxonomists, geneticists, and horticulturists are able to find workable rules that are commonly acceptable.

In the meantime, however, the increased interest in hybrids as they relate to genetic and population studies makes their proper identification as important as that of species. The fact that improper identification of hybrid or supposed hybrid material also affects directly the proper identification of the species in a given genus is shown by the above example of *S.* ×*Catesbaei.*

Designation by formula rather than name seems most expedient in the case of natural hybrids without any horticultural value or background. When natural hybrids also have horticultural counterparts, and horticultural names get into the literature dealing with such plants, it seems best to recognize the name and treat it according to the rules of botanical nomenclature. In the case of *Sarracenia* hybrids many horticultural names are already in the literature and should be properly dealt with. All but three of the natural hybrids considered in this paper have been named previously, some by botanists, some by horticulturists. For the sake of uniformity and because of their potential horticultural role these three hybrids are given names and not referred to by formulae alone.

The present paper is an attempt not only to consolidate numerous scattered taxonomic references concerning *Sarracenia* hybrids, thus making any future rules of nomenclature regarding hybrid or horticultural plants more readily applicable to this genus, but also to present figures and descriptions to make their identification more accurate. In addition to previously described hybrids, one new natural hybrid is described, and descriptions are given for several hybrid Sarracenias that have previously been reported or listed in the literature without description.

Delay (1951) lists chromosome counts of only two *Sarracenia* hybrids, *S.* ×*Mitchelliana* and *S.* ×*Moorei*, both of which have an unreduced chromosome number of 26 (which is also the unreduced number found in all species of this genus). Further studies on the cytology of all natural *Sarracenia* hybrids are proposed, as well as genetic or statistical studies of some of these hybrids which occur in large enough numbers to make such studies practical.

BRIEF HISTORY

Elliott (1824) described as a new species of *Sarracenia* some plants collected in South Carolina by Dr. Macbride. From its description and later observations, which included Elliott's material, the "new species," *S. Catesbaei*, was determined to be a hybrid between *S. purpurea* and *S. flava* and was recognized as such by Macfarlane (1908). In the meantime, however, the plant's identity was thoroughly confused, as the following chronology will show. In Eaton's Manual of Botany (1836) the plant is considered a species, *S. Catesbaei;* Croom (1848) considered it "hardly a variety of S. flava;" Decaisne (1851) named it under *S. flava;* later the name *S. flava Catesbaei* was used by Mohr (1897) to refer to some plants

growing along Little River in northeastern Alabama; Mohr (1901) soon raised these plants to specific rank and, thinking them to be distinct from *S. flava* and similar to those described by Elliott, called them *S. Catesbaei;* Harper (1903) incorrectly applied the name *S. Catesbaei* to some hybrids between *S. Drummondii* and *S. flava;* in an article on the history of *S. Catesbaei*, Macfarlane (1904) considers the name to apply to the atypical *S. flava* growing around Mobile, Alabama, plants which today are recognized as constituting the species *S. Sledgei;* when Macfarlane (1908) monographed the genus for *Das Pflanzenreich*, he had recognized the hybrid nature of *S. Catesbaei* and described the Mobile plants as a new species—*S. Sledgei*. Mohr's plants from along Little River were placed under *S. flava* by Macfarlane (1908), and were not recognized and properly described as a distinct species until 1933, when they were described as *S. oreophila* by Wherry.

No other name in the genus has had such a varied history as *S. Catesbaei*, but the synonomy of the various hybrids will show the effect of a brief period of horticultural popularity on the nomenclature of the genus. A similar effect may be noted in the case of *S. flava* as it is treated by various authors.

The first artificial *Sarracenia* hybrid to flower was exhibited and described in 1874 by Dr. David Moore of the Glasnevin Botanic Gardens, Dublin. Although four to seven years are required to raise a *Sarracenia* hybrid to flowering size, another artificial hybrid, *S. flava* × *S. purpurea*, was reported within a week. Hence, horticultural interest in these plants must have started around 1870. This interest increased until about 1886, when these plants lost general popularity as suddenly as they had gained it.

Moore's hybrid, later treated under the name *S. Moorei* × by Masters (1881), was the product of a cross between *S. flava* and *S. Drummondii;* and its "exactly intermediate rank between the parent plants" was noted by Moore (1874). Form and coloration were not the only intermediate characters (Plate 4, fig. 2b), as may be surmised by Moore's statement just quoted, but also such characteristics as the type of second growth and duration of the pitchered leaves. This intermediate character of the hybrids, occasionally altered in the direction of one or the other of the parents by the direction of the cross or natural backcrossing, has been noted by all who have observed these plants, and Russell (1919) found the intermediate nature of *Sarracenia* hybrids to extend even to cellular detail.

One of the few references to these hybrids' not being intermediate in all respects is found in *Das Pflanzenreich* (**4:** pt. 110, p. 35) where Engler, discussing the artificial hybrid *S. Drummondii* × *S. minor* (which should have red and yellow, or pinkish petals), says, "Interessant ist, dass einige der Pflanzen tief scharlachrote Blumen bringen, wie *S. Drummondii*, während andre die gelbe Blüte von *S. variolaris* zeigen."

There are other references, by Oliver (1899) and Rolfe (1900), that indicate that the direction of the cross seems to influence hybrid form in some cases, the hybrid not showing completely intermediate characters, but resembling more closely the female parent. This problem may be more widespread than is realized from the small amount of hybrid *Sarracenia* material available for study. There

seems to be good evidence of it in at least two cases and the topic will be treated further under the hybrids involved (see 3. *S. purpurea* × *minor* and 5. *S. purpurea* × *rubra*).

Natural counterparts are possible of twelve of the numerous *Sarracenia* hybrids produced and described by horticulturists of the late nineteenth century. Eleven of these have subsequently been reported by American workers, and Manda (1892) mentions several instances of European horticulturists' receiving natural *Sarracenia* hybrids in shipments of the native species from the United States. These eleven are included in the total of thirteen natural hybrids thus far on record.

DISTRIBUTION AND IDENTIFICATION

Geographical isolation appears to be the primary positive isolating mechanism in the genus *Sarracenia;* accordingly, of the eight species of the genus, only the isolated *S. oreophila* has not been found to be involved in at least one natural hybrid. At the other extreme, the wide-ranging *S. purpurea* is found to grow over part or all of the ranges of six other species, and all the six different natural hybrids possible between *S. purpurea* and these species have now been found.

The greatest concentration of *Sarracenia*, both in number of individual plants and in number of species, occurs in the southern half of Georgia and Alabama and in northern Florida. It is not surprising, therefore, that many of the possible natural hybrids occur in this area.

Table 1 will summarize briefly the potential and actual distribution of natural *Sarracenia* hybrids. It might be well to note that certain species are rare or have very limited range in some states, and that although hybrids may occur, the chances of discovering them are rather small. Thus, no natural hybrids are reported from Mississippi even though nine would be expected to occur there insofar as geographical and ecological considerations are concerned.

Seasonal isolation, which seems to restrict the interbreeding of *S. psittacina* more than that of other species, is by no means absolute, as is geographic isolation. The following extremely general table, prepared from field notes and herbarium data, will show the approximate blooming periods of the different species of *Sarracenia* in the Georgia-Alabama-Florida area previously mentioned. In the nine cases where seasonal isolation does occur between sympatric species it is often so weak that any "unseasonable" weather or even normal "local conditions" would tend to break it and, in the apparent absence of any genetic isolating mechanisms, allow the species to hybridize.

Identification of *Sarracenia* hybrids by the use of keys would be difficult because of their intermediate character. The only character possessed by a hybrid and usually not possessed by either of its parents is the red and yellow, or pinkish, color of the flowers of hybrids resulting from crosses between red-flowered and yellow-flowered species. The exception is *S. rubra*, which often has some yellow-green on the inner surface of the petals, but otherwise has maroon flowers. Except for the three species, *S. oreophila*, *S. flava*, and *S. Sledgei*, leaf characters are of primary importance in the separation and identification of species of *Sarracenia*.

TABLE 1

The distribution of natural Sarracenia *hybrids*

	VA.	N.C.	S.C.	GA.	FLA.	ALA.	MISS.
1. *S. purpurea* × *flava**	⊗	⊗	⊗	×	⊗	⊗	
S. ×*Catesbaei*							
2. *S. purpurea* × *Drummondii*				×	⊗	⊗	×
S. ×*Mitchelliana*							
3. *S. purpurea* × *minor*		⊗	×	×	×		
S. ×*Swaniana*							
4. *S. purpurea* × *psittacina*				×	×	×	×
S. ×*Courtii*							
5. *S. purpurea* × *rubra*		⊗	×	×	×	×	
S. ×*Chelsoni*							
6. *S. purpurea* × *Sledgei*						⊗	×
S. ×*ezornata*							
7. *S. Drummondii* × *flava*				⊗	⊗	⊗	
S. ×*Mooreana*							
8. *S. Drummondii* × *minor*				×	×		
S. ×*excellens*							
9. *S. Drummondii* × *psittacina*				×	⊗	×	×
S. ×*Wrigleyana*							
10. *S. Drummondii* × *rubra*				×	⊗	⊗	×
S. ×*Readii*							
11. *S. Drummondii* × *Sledgei*						⊗	×
S. ×*areolata*							
12. *S. minor* × *flava*		×	⊗	⊗	×		
S. ×*Harperi*							
13. *S. minor* × *psittacina*				⊗	×		
S. ×*formosa*							
14. *S. minor* × *rubra*		⊗	×	⊗	×		
S. ×*Rehderi*							
15. *S. rubra* × *flava*		⊗	×	×	×	×	
S. ×*Popei*							
16. *S. rubra* × *psittacina*				×	×	×	×
17. *S. rubra* × *Sledgei*						×	×
18. *S. psittacina* × *flava*				×	×	×	
19. *S. psittacina* × *Sledgei*						×	×

× = Geographically possible, but unreported to date.

⊗ = Natural hybrid reported from this state.

* In the absence of more precise information as to the direction of the cross the International rules call for the names of the two parents involved in a cross to be listed alphabetically in the formula designating a given hybrid. In this table, however, the formulae are not intended as "names" but in most cases are given only to facilitate comparison of the various hybrids, and are therefore presented in a manner which the author feels is most satisfactory for this purpose.

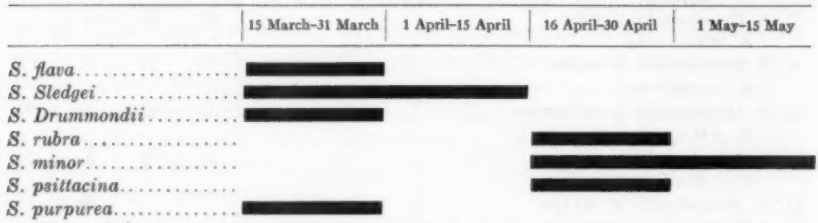
Since the three species just listed do not hybridize (geographic isolation), any potential hybrids must have parents with relatively different leaf form and color. Therefore, if the seven possible parental types are known, identification of first-generation hybrids is not too difficult except in the case of hybrids produced by

species which cross with both *S. Sledgei* and *S. flava;* these must usually be separated on the basis of geography. Walcott (1935) and others have illustrated the various species of *Sarracenia*, and an attempt is made here to furnish not only brief descriptions of the natural hybrids, but photographs or figures which will be of far more value in the identification of doubtful specimens than would the descriptions alone.

Hybrid *Sarracenia* material for this study was kindly loaned by the directors of the following herbaria: United States National Herbarium (US), University of Pennsylvania (PENN), Philadelphia Academy of Science (PH), University of North Carolina (CNC), and the New York Botanical Garden (NY). The abbreviations for these herbaria follow those proposed by Lanjouw (1939).[2]

TABLE 2

The approximate blooming periods of species of Sarracenia *in Georgia, Alabama, and Florida*



With the exception of *S.* ×*Catesbaei* no mention of any type specimens has been found for the various *Sarracenia* hybrids; therefore types, lectotypes, or neotypes are designated wherever appropriate in the following treatment of these hybrids. Since the primary considerations here are the natural *Sarracenia* hybrids and not the horticultural ones, it was felt that examination of the early horticultural *Sarracenia* material of some European herbaria would not be relevant. Even so, if inquiry should turn up horticultural specimens of *S.* ×*excellens* and *S.* ×*Wrigleyana*, they should be designated as lectotypes from cultivation (as was done in the case of *S.* ×*Courtii*, in lieu of a collection of this plant as a natural hybrid).

1. *S. flava* × *purpurea*

Sarracenia ×**Catesbaei** (Elliott) Bell, stat. nov.

S. Catesbaei Elliott, Sketch of the Botany of S. C. and Ga. **2:** 11. 1824; *non* Macfar. Contr. Bot. Lab. Univ. Penn. **2:** 426, 1904.
×*S. Stevensii* Masters, Gard. Chron. ser. 2, **1:** 738. 1874.
×*S. Williamsii* Hort. ex Masters, Gard. Chron. ser. 2, **2:** 49. 1874.
S. Stevensi × Masters, Gard. Chron. ser. 2, **16:** 40. 1881.
S. Williamsi ×, Hort. Williams ex Masters, Gard. Chron. ser. 2, **16:** 40. 1881.

[2] Since this paper was submitted a recent revision of this work (Lanjouw, J. and F. A. Stafleu. 1952. The herbaria of the world. Regnum vegetabile **2:** 111) gives the abbreviation for the University of North Carolina Herbarium as NCU instead of CNC.

S. porphynoneura Hort. ex Masters, Gard. Chron. ser. 2, **17:** 812. 1882.
S. Atkinsoniana S. G. Garden **28:** 219. 1885.
S. Patersoni S. G. Garden **28:** 218. 1885.
S. Wilsoniana S. G. Garden **28:** 218. 1885; *non* Robinson, Garden **24:** 447. 1883.
S. Tolliana S. G. Garden **28:** 219. 1885; *non* Robinson, Garden **24:** 447. 1883; *non* Nicholson, Dict. Gard. **3:** 366. 1887.
S. Wilsonii Manda, Roy. Hort. Soc. Jour. **15:** 143. 1892.
S. Eblana Hort. Jour. Roy. Hort. Soc. **21:** ccvii. 1897.
S. porphyroneura Macfar. *in* Bailey, Stand. Cycl. Hort. **6:** 3081. 1917.

Plants intermediate between parents. Leaves pitchered, green to heavily shaded and veined with dark red-purple, erect or suberect, elongate, 1.5–4.5 dm. tall (usually 2.5–3.5 dm.), widely tapered from base to lip of pitcher, slightly distended below the orifice, adaxial wing widest below the middle; erect, prominent, reniform-cordate hood 4.5–14.5 cm. broad (usually 5.5–9.5 cm.), reticulated with dark maroon veins, adaxial surface hispid, the white hairs numerous and all pointing toward the orifice, abaxial surface pubescent. Scape as long as or longer than the leaves. Flowers large, to 17 cm. across; sepals red-brown, or with green inner surface, ovate-oblong, obtuse; petals maroon outer surface with red-yellow inner surface, or rose with yellow margins.

Type locality: "along the margins of rivulets amidst the high sand hills of Chesterfield district in S. Carolina."

Possible range: In association with parent species from south-central Virginia through eastern North Carolina and South Carolina to below Charleston, and again from the southwest corner of Georgia and northern Florida westward to the vicinity of Mobile, Alabama.

Representative specimens: VIRGINIA. Petersburg, Dinwiddie Co., *Fernald & Long 6214* (PENN); Dahlia, Greensville Co., *Fernald & Long* (US 1810769). NORTH CAROLINA. Dunn, Harnett Co., *Browne* (CNC ?); Wilmington, Brunswick Co., *Jones* (PENN 71650). SOUTH CAROLINA. Chesterfield Co., *Macbride* (CHARL.: type). FLORIDA. Quincy, Gadsden Co., *Wherry* (US 1466415); Ponce de Leon, Holmes Co., *Macfarlane* (PENN 38475).

The extensive areas of coincident range of the two parent species make this one of the *Sarracenia* hybrids most commonly collected. No record has been found of these hybrids occurring in the great numbers of those between *S. Drummondii* and *S. Sledgei*, but Macfarlane (1908) reported finding 117 plants of *S.* ×*Catesbaei* in one day in Holmes County, Florida.

Offspring of this cross often seem to show heterosis (see Plate 7, fig. 3), but despite their very robust appearance the leaves do not have the almost evergreen quality of *S. purpurea*. The large erect hood of the hybrid may be undulate but it is not closely, if at all, curved around the orifice of the pitcher, as it is in *S. purpurea;* however, the hispid nature of the hood and the red coloration in the flowers are obvious contributions of this parent. Height and taper of the leaf, the somewhat cordate hood, and the yellow tints of the petals are from the *S. flava* parent.

2. *S. Drummondii* × *purpurea*

Sarracenia ×Mitchelliana (Nichols.) Bell, stat. nov.

S. Mitchelliana Nichols. Dict. Gard. **3:** 365. 1887.
S. Wilsoniana Hort. ex Wm. Robinson, Garden **24:** 447. 1883; *non* S. G. Garden **28:** 218. 1885.
S. Tolliana Hort. ex Wm. Robinson, Garden **24:** 447. 1883; *non* S. G. Garden **28:** 219. 1885.
S. Patersonii Hort. Macfar. Das Pflanzenreich **4:** pt. 110. 1908, *non* S. G. Garden **28:** 218. 1885.

Plants intermediate between parents. Pitchered leaves erect or nearly so, green, heavily shaded and/or veined with deep red and mottled with white around orifice, 2–6 dm. tall (usually 2.2–3.5 dm.), widely tapered from base to lip of pitcher, slightly distended below the orifice, adaxial wing linear, widest near middle; reniform-cordate hood erect, often strongly undulate, 5–9.5 cm. broad, reticulated with darker red veins and blotched with white or pink, abaxial surface pubescent, adaxial surface hirsute-hispid, some of the white hairs reaching a length of 2 mm. and all pointing toward the orifice. Scape longer than leaves. Flowers large, 10 cm. across; sepals 3–4 cm. long, ovate, maroon, tinged with green on inner surface; petals 4–5 cm. long, dark maroon.

Type locality: Bog, 2.5 miles west of Florida state line by highway U. S. 90, Baldwin Co., Alabama. 1948.

Possible range: In association with parent species in the southwestern corner of Georgia and northern Florida from Tallahassee westward through southern Alabama and Mississippi to the Louisiana state line, where it reaches the Gulf of Mexico.

Specimens examined: ALABAMA. Bay Minette, Baldwin Co., *Bell 548* (CNC: neotype); Theodore, Mobile Co., *Bell 1223* (CNC).

There are records of other collections of this plant. Macfarlane (1908) seems to have been the first to collect it near Bay Minette, Alabama, in 1905. These plants must have been kept as living specimens since they are not included in Macfarlane's collections. Harper (1918) found it in Walton County, Florida, in 1911, but no specimens from this locality have been seen. Unlike *S.* ×*Catesbaei*, both parents of *S.* ×*Mitchelliana* have hoods with hirsute-hispid inner surfaces, and the floral differences of the two parents of *S.* ×*Mitchelliana* are so slight that they cannot be expected to be detected as "intermediate" in the hybrid. However, some leaf characters of the two parents are so distinct that accurate determination of the parentage is easy. The slightly distended pitchers and the large, rather erect hood immediately suggest *S. purpurea* (Plate 2, fig. 2), while the height of the leaves and the unmistakable white splotches around the orifice and on the hood limit the possible second parent to *S. Drummondii*.

3. *S. minor* × *purpurea*

Sarracenia ×Swaniana (Wm. Robinson) Bell, status novus.

S. Swaniana Hort. ex Robinson, Garden **24:** 447. 1883.
?*S. Flambeau* Hort. ex Macfar. in Das Pflanzenreich **4:** 110: 38. 1908.
S. Swainiana Eliot et Stauffer, Wild Flower Mag. **27:** 40. 1951.

Plants intermediate between parents. Pitchered leaves suberect, green, reticulate or shaded with dark red (especially plants growing in full sun), lightly maculated with a few white opaque blotches at the base of the hood, 1.5–2.5 dm. tall, tapered from base to lip of pitcher, slightly distended below the orifice, adaxial wing linear, more or less undulate, broadest just below the middle, thus complementing the gentle curve in the hollow body of the leaf; cordate-reniform hood slightly arched over orifice, 4–6 cm. broad, more or less heavily marked with maroon, abaxial surface pubescent, adaxial surface hirsute-hispid, the hairs pointing toward the orifice. Scape longer than the leaves. Flowers intermediate to smaller than those of either parent species; sepals red-green; petals red, shading to pink at the edges and pale yellow-pink on the inner surface.

Type locality: Damp savannahs about 12 miles southwest of Shallotte, Brunswick Co., North Carolina. 1952.

Possible range: In association with parent species in southeastern North Carolina and the coastal half of South Carolina as far south as Charleston, and again in limited areas of southern Georgia and northern Florida west to Tallahassee.

Specimen examined: NORTH CAROLINA. Shallotte, Brunswick Co., *Rehder* (CNC: neotype).

No published record of this plant as a natural hybrid existed until last year, when Eliot and Stauffer (1951) published the name and parentage in a list of plants shown them by Mr. Henry Rehder of Wilmington, N. C. Mr. Rehder had previously discovered about thirty of the plants in Brunswick County and had sent not only descriptions and fresh material, but also color photographs, to the present author, for verification of the identification and for use in this paper.

The change in leaf coloration of *S.* ×*Swaniana*, from green in shaded plants to dark red in plants exposed to full sun, is a very interesting parallel to the condition found in *S. purpurea* and noted by Macfarlane (1908) and Bell (1949). Doubtless, this character comes entirely from the *S. purpurea* parent, as no color:shade relationship has ever been observed in *S. minor*. Other characters of a more constant nature which are contributed by *S. purpurea* are the wide, cordate hood, the slightly distended pitcher, the undulate lateral wing, and the red coloration in the flower. The "arch" at the base of the hood (Plate 3, figs. 1–2), the translucent spotting, and the yellow floral coloration are due to *S. minor*.

From the material and descriptions furnished by Mr. Rehder it appears that either the direction of the cross influences the appearance of the hybrid, or that some of the hybrid plants have back-crossed to *S. purpurea*. In view of the intermediate nature of some of the material and the weak (no translucent areolations) but definite (hood shape and wing) influence of *S. minor* in other material, the backcross hypothesis seems a likely possibility at the present time. However, the leaf of *S.* ×*Swaniana* pictured by Nicholson (1887) shows more *S. purpurea* characters than would be expected in a truly intermediate leaf; and a comment in *The Garden* (p. 218, vol. **28**, 1885) says of the leaves: ". . . they

partake most of the aspect of *S. purpurea*, but they are more erect, funnel-shaped, slightly incurved, . . ." With more observations the first hypothesis may prove to be correct.

4. *S. psittacina* × *purpurea*

Sarracenia ×Courtii Masters, Gard. Chron. ser. 2, **16:** 381. 1881.

S. Courtii × Masters, Gard. Chron. ser. 2, **16:** 381. 1881.

Plants intermediate between parents. Described in the *Gardeners' Chronicle* (p. 381, ser. 2, **16,** 1881) as ". . . a cross between S. purpurea, as the seed parent, of which it has the deep wing, and S. psittacina as the male parent, of which it has the general form and habit, having small, spreading pitchers of a deep red colour, deeply winged, and with a concave, cowl-like lid, as in psittacina." (Plate 6, fig. 4.)

Type locality: Type from cult., England.

Possible range: In association with parent species in southwestern Georgia, northwestern Florida, and across southern Alabama into southeastern Mississippi.

Specimen examined: No native material. One horticultural specimen from England, *Harrison* (US 2997: lectotype).

Russell (1919, p. 9) listed this cross in a paragraph noting "other natural hybrids reported by various collectors." The two references cited were both works of Macfarlane. The first was not seen, but the second (later) reference was to Macfarlane's treatment of *Sarracenia* in *Das Pflanzenreich* and was carefully reviewed. In this, Macfarlane says (p. 22), "Curiously enough, though the writer has explored many miles of territory in which *S. purpurea* and *S. psittacina* grew side by side in great abundance, he has failed to note even one example of the hybrid."

In all probability *S.* ×*Courtii* has yet to be discovered in its native habitat. As regards the *S. psittacina* parent, this may be due to seasonal isolation, which may be effective in the rather uniform climate and relatively small range available to this hybrid. Plants of *S. psittacina* in very early bud were collected on March 21, 1948, near Theodore, Alabama; yet plants of *S. purpurea* in the same field were in full bloom. *S. psittacina* coincides in range with all or parts of the ranges of six other species of *Sarracenia*, and it might be expected to hybridize with these species without difficulty. However, only one natural cross involving *S. psittacina* has been reported in the literature and documented by herbarium material (see *S. minor* × *psittacina*).

5. *S. purpurea* × *rubra*

Sarracenia ×Chelsoni Masters, Gard. Chron. ser. 2, **8:** 600. 1877.

×*S. Chelsoni* Hort. Veitch ex Masters, Gard. Chron. ser. 2, **8:** 600. 1877.

Plants intermediate between parents. Leaves suberect, curving slightly from the base, green, reticulated or shaded with dark red, 1.5–4 dm. tall, more or

less broadly tapered from decurved base to lip of pitcher, slightly distended below the orifice, adaxial wing slightly undulate, broadest below the middle; hood erect, reniform or more usually cordate, expanded, 4–6 cm. broad, margins more or less undulate, adaxial surface hispid, the long white hairs all pointing toward orifice, abaxial surface pubescent. Scape much longer than the leaves. Flowers maroon, without scent; the sepals brownish-maroon, ovate-oblong, slightly or moderately recurved; petals maroon, or with yellow-green edges or inner surface, obovate.

(The hybrid *S. purpurea* × *S. rubra* f. *Jonesii* is also considered in this taxon. This accounts for some of the larger upper limits on a few of the measurements. See Plate 2, fig. 3.)

Type locality: Eastern shore of Scotland Lake, Scotland Co., North Carolina.

Possible range: In association with parent species locally in the mountains of western North Carolina, more generally in southeastern North Carolina, the coastal half of South Carolina, southern Georgia and northwestern Florida west through southern Alabama into southeastern coastal Mississippi.

Specimens examined: NORTH CAROLINA. Etowah, Henderson Co., *Wherry* (US 1469599), *Clements* (CNC); Scotland Co., *Wellman* (CNC 30805: neotype).

Oliver (1899) mentions a collection of this natural hybrid from "near Wilmington, N. C." Wherry has also seen the plant in that general part of North Carolina, but none seem to have been observed by Macfarlane or Harper, who have collected *Sarracenia* rather extensively in the other areas in which *S.* ×*Chelsoni* might be expected.

It is rather surprising that the relatively faint yellow-green sometimes observed in the petals of *S. rubra* occasionally shows up in the flowers of this hybrid, since the primary and predominant maroon color of the flowers of the two parents would lead one to expect entirely maroon flowers in the hybrid. The sepal characters of the two parent species are quite different, as described and shown in comparative photographs by Bell (1949). These characters were at first intermediate in the plant of *S.* ×*Chelsoni* that bloomed in the Botany Department greenhouse at the University of North Carolina; but, as the flower aged, the sepals became more recurved and finally appeared, in this respect at least, more like those of the *S. rubra* parent. A figure of *S.* ×*Chelsoni* in *The Gardeners' Chronicle* ser. 2, **13:** 725, 1880, and another in a later issue (ser. 2, **15:** 821, 1881) show the leaves of this hybrid to resemble those of *S. purpurea* (the pollen parent!) much more closely in hood characteristics than was actually observed in the aforementioned natural hybrid (direction of cross unknown) growing under "cultivation" in the greenhouse, (Plate 4, fig. 1). The adaxial wing seems quite variable in this hybrid. The wings of leaves figured in the *Gardeners' Chronicle* and those of Wherry's specimen from western North Carolina are quite narrow, while those shown in the photograph of the eastern North Carolina plant are obviously very broad and somewhat undulate.

This brings up the question of the influence of the direction of the cross previously mentioned. It would seem that if the seed parent had the greater effect on the hybrid offspring, the cross onto *S. rubra* should give offspring of which

the leaves had rather apiculate hoods and narrowly linear wings. This, however, does not appear to be the case; for, when *S. rubra* is known to be the seed parent, the linear wing is present in the hybrid, but the reniform hood approaches that of *S. purpurea* (Plate 7, fig. 4). When the hood is more cordate-apiculate (Plate 4, fig. 1), the adaxial wing is broad and undulate. Speculation, however, cannot solve genetic problems. So experiments and further observation are needed. At present we may say that in some cases the range of the "intermediate" category, as concerns some *Sarracenia* hybrids, is unusually broad. The direction of the cross may be a contributing factor.

6. *S. purpurea* × *Sledgei*

Sarracenia ×exornata (S. G.) Bell, stat. nov.

S. exornata S. G. Garden **38:** 218. 1885.
S. exoniensis Hort. Veitch ex Roy. Hort. Soc. Jour. **24:** 26. 1900.

Plants intermediate between parents. Leaves green to heavily shaded or veined with dark red, erect or suberect, elongate, 1.5–3.8 dm. tall (usually 1.7–2.5 dm.), widely tapered from base to lip of pitcher, slightly distended below the orifice, adaxial wing widest below the middle; erect, prominent reniform-cordate hood 4.5–8.2 cm. broad (usually 4.7–6.6 cm.), slightly undulate, reticulated with dark maroon veins or solid maroon, adaxial surface hispid, the white hairs pointing toward the orifice, abaxial surface pubescent. Scape longer than leaves. Flowers up to 10 cm. across; sepals red-brown or tinged with green on outer surface, greenish inner surface, ovate, obtuse; petals maroon outer surface with red-yellow inner surface, or rose with yellow margins.

Type locality: Theodore, Mobile Co., Alabama.

Possible range: In association with parent species in the extremely limited range of coastal Alabama and Mississippi from the area of Mobile westward to the Louisiana state line (and possibly on to the Mississippi River).

Specimens examined: ALABAMA. Spring Hill, Mobile Co., *Graves* (US 1071688); Theodore, Mobile Co., *Pennell* (PENN 58126: neotype), *Bell 542* (CNC).

Sarracenia ×exornata and *S. ×Catesbaei* have one parent species in common and the similarity between the two species, *S. Sledgei* and *S. flava*, respectively, which are the other elements in these two hybrids, make them almost identical. The only difference is probably one of size, for *S. Sledgei* is not as gross in size and form as *S. flava*, and as a result *S. ×exornata* is not quite as robust in appearance as *S. ×Catesbaei*. This may be noticed when several specimens of each hybrid are placed together for comparison, but would hardly be detected in individual collections. The best (or only) separation of these two hybrids must depend on the area in which they are found, since *S. flava* does not seem to reach Baldwin or Mobile counties in Alabama where *S. Sledgei* has its eastern limit.

The name "S. exornata," in *The Garden* (**28:** 218), was applied originally to "a hybrid between S. purpurea and S. crispata." *Sarracenia crispata*, however, is now considered a synonym of *S. Sledgei*. So the name *S. ×exornata* is applicable to the hybrid between *S. purpurea* and *S. Sledgei*.

7. *S. Drummondii* × *flava*

Sarracenia ×Mooreana (Veitch) Bell, stat. nov.

S. Mooreana Veitch, Gard. Chron. ser. 2, **7**: 425. 1877.
S. Moorei × Masters, Gard. Chron. ser. 2, **16**: 40. 1881.
S. exculta S. G. The Garden **28**: 219. 1885.
S. exculta Nichols. Dict. Gard. **3**: 364. 1887. (Listed as *S. exsculta* Nicholson, in Kew Index Suppl. **1–2**: 380. 1886–95.)
S. Tolliana Nichols. Dict. Gard. **3**: 366. 1887; *non* Hort. Garden **24**: 447. 1883; *non* S. G. Garden **28**: 219. 1885.
S. Mandaiana Hort. ex Masters, Gard. Chron. ser. 3, **13**: 455. 1893.
S. exsculpta Nichols. Dict. Pratique Hort. **4**: 641. 1896.
S. Brucei Hort. Bruce ex Roy. Hort. Soc. Jour. **39**: cxxxiv. 1913.

Plants intermediate between parents. Summer leaves erect, elongate, trumpet shaped, 3–9 dm. tall (usually 4–6.5 dm.), narrowly tapered below, expanding evenly to lip of pitcher above, green, marked or shaded with red, the areoles around the orifice pale gray-green or white, adaxial wing linear, narrow; winter or late summer leaves ensiform or with recurved tips, up to 5.5 dm. tall, green with few or no markings, persistent; hood of summer leaves suberect, reniform-cordate to suborbicular, 4–10.5 cm. broad (usually 5–8 cm.), narrowed and reflexed at base, white or pale gray-green often strongly marked with prominent, deep-red veins, the "throat" or narrowed base usually banded adaxially with iridescent red-purple, hood margins revolute or undulate, adaxial surface hispid, the white hairs pointing toward the orifice, abaxial hood-surface pubescent. Scape as long or longer than summer leaves. Flowers large, 10 cm. or more across; sepals ovate to oblong, obtuse, brown-green or maroon on outer surface, greenish within; petals maroon outer surface, red-yellow within or pinkish with yellow margins.

Type locality: Sandy bog southeast of Americus, Sumter Co., Georgia. 1901.

Possible range: In association with parent species in limited areas of southwestern Georgia, more extensively in northwestern Florida and southern Alabama westward to the western limit of the range of *S. flava*, east of Mobile.

Representative specimens: GEORGIA. Americus, Sumter Co., *Harper 1021* (US 400378: neotype). FLORIDA. Crestview, Okaloosa Co., *Macfarlane* (PENN 49351–49354); Milligan, Santa Rosa Co., *Macfarlane* (PENN 49339–49340). ALABAMA. Geneva, Geneva Co., *Harper 102* (NY).

This large and colorful natural hybrid was one of the first to be found, according to Harper (1918), who mentions it as an item advertised in the 1893 catalogue of Pitcher and Manda, New Jersey florists, under the name of *Sarracenia Mandaiana*. The plant was illustrated and its hybrid nature correctly assumed, since it was said to be growing with both *S. flava* and *S. Drummondii*. Harper earlier (1903) mentions seeing the plant near Americus, Georgia, in 1895, but the earliest collection of it seems to have been in 1901 at that locality. At that time Harper considered the plant to be Elliott's *S. Catesbaei*, or possibly a hybrid between *S. Drummondii* and *S. rubra*, which were growing in the same locality. The possibility that *S. flava* was one parent was discounted by Harper, who said this species was not known to occur within twenty-seven miles of this locality. Possibly *S. flava* had died out at this station, or perhaps geographically

closer populations of this species remained undiscovered. At any rate the collection *Harper 1021* (US 400378), cited by Harper (1903), is most certainly *S.* ×*Mooreana*.

In general form, the pitchers of the two parent species of *S.* ×*Mooreana* are rather similar, but the apiculate, revolute hood and crimson "throat" of *S. flava* are immediately noticeable in the hybrid, as is the yellow coloration in the fresh flowers, when they are available. The unmistakable white spots on the hood and around the orifice can only indicate *S. Drummondii* (Plate 4, fig. 2b and Plate 7, fig. 2). Other characters contributed to the hybrid by this latter parent are the undulate hood margins, the hispid adaxial surface of the hood, much of the extremely heavy red venation of the upper part of the leaf, and, of course, the maroon tint or color of the flowers.

The status of *S.* ×*Mooreana* as the first artificial hybrid in the genus has been mentioned previously.

8. *S. Drummondii* × *minor*

Sarracenia ×**excellens** (Nichols.) Bell, stat. nov.

S. excellens Nichols. Dict. Gard. **3**: 364. 1887.
S. cantabrigiensis Lynch ex Zahn in Gartenwelt **12**: 82. 1907.

Plants intermediate between parents. Described by Nicholson (1887, p. 364) as "1. green, becoming stained with close reticulations of purplish-red near the upper end, the pallid spots reddish externally; lid roundish, arching, and, with the upper part of the tube, suffused and mottled with dark red. Habit erect. Parents: *S. variolaris* and *S. Drummondii alba*."

Type locality: No collections of this hybrid have been found.

Possible range: In association with parent species in the extremely limited range of portions of southwestern Georgia and northern Florida approximately fifty miles north and east of Tallahassee.

The originally small potential area of this hybrid has doubtless been made even smaller by the inroads of civilization. So even though the parent species could possibly bridge the gap of two weeks' seasonal isolation, their geographic contact is so tenuous that it is entirely possible that this interesting natural hybrid, so far unreported, will never be found under natural conditions.

9. *S. Drummondii* × *psittacina*

Sarracenia ×**Wrigleyana** (S. G.) Bell, stat. nov.

S. Wrigleyana S. G. Garden **28**: 219. 1885.

Plants intermediate between parents. Described in the above reference as the product of "a cross between S. psittacina and S. Drummondi alba, and while the charming white mottlings peculiar to this lovely upright-growing kind are conspicuously preserved and even improved in the offspring, inasmuch as they extend half way down the pitchers, the latter in shape forcibly remind one of their descent from S. psittacina. It may be said that the pitchered leaves are intermediate between the two parents. They grow from 12 inches to 15 inches high and are slightly curved, dilated in the centre, and, besides the white mot-

tlings already noticed, are further ornamented with numerous fine reticulations of a lively reddish tinge; their base, very narrow, is also of a very peculiar yellowish green and very attractive in colour."

Type locality: No collections of this hybrid have been found.

Possible range: In association with parent species in limited areas of southwestern Georgia and then more widely in northwestern Florida and southern Alabama west into southeastern Mississippi.

Although no collection in nature of this plant has been reported and there are no herbarium specimens of *S.* ×*Wrigleyana*, the relatively large area common to both parent species makes future collection of this plant a much more distinct possibility than could be hoped for in the case of *S.* ×*excellens*, though the seasonal isolation factor is the same in both cases. Indeed, in a letter of December 7, 1949, Dr. E. T. Wherry mentions once finding a plant of *S. Drummondii* × *S. psittacina* in a pasture in Florida. Unfortunately, the plant had been trampled by cattle and was not collected, but perhaps others will be found.

10. *S. Drummondii* × *rubra*

Sarracenia ×Readii hybr. nov.

?*S. Farnhami* Hort. ex Macfar. Pflanzenreich **4:** 110: 32. 1908. Nomen nudum.
S. Readi Macfar. ex Bell, Journ. Mitchell Sci. Soc. **65:** 162. 1949. Nomen nudum.

Sarracenia ×Readii, hybrida naturalis inter S. Drummondium et S. rubram nova foliis erectis tubulatis e basi ad foramen versus diametro gradatim accrescentibus basi viridibus ad apicem versus rubro-reticulatis circum foramen areolis albis ornatis 2–3 dm. altis ala adaxiali anguste lineari infra medium latissima cucullo suberecto cordato apiculato basi constricto albo venis rubris reticulato marginis leviter undulatis superficie adaxiali hispida ea abaxiali hirsuta floribus mihi ignotis.

Plants intermediate between parents. Leaves erect, tubular, 2–3.2 dm. tall, narrowly tapered from base to lip of pitcher, green below, veined with red above with conspicuous white areoles around orifice, adaxial wing linear, narrow, widest below middle; hood slightly arched over orifice, cordate, apiculate, 1.4–2.5 cm. broad, narrowed at base, white, strongly marked with dark red veins, margins slightly undulate, adaxial surface hispid, the hairs pointing toward the orifice, abaxial surface hirsute. Flowers unknown.

Type locality: Deer Park, Washington Co., Alabama. 1913.

Possible range: In association with parent species in limited areas of southwestern Georgia, more extensively in northwestern Florida and southern Alabama westward into southeastern Mississippi.

Specimen examined: ALABAMA. Deer Park, Washington Co., *Reade* (PENN 57859: type).

This plant was collected by L. H. Reade and evidently sent to Macfarlane, who labeled it "Sarracenia Drummondii × S. rubra = S. Readi" and added the note, "a rare hybrid, found also by me at Milligan, Fla." The above name for this hybrid was never published by Macfarlane, but it was used as a "horticultural" name by Bell (1949).

Although no flowers of this plant have been reported, it should be safe to assume that they would be intermediate between those of the parent species in size and form. Since both parent species are maroon flowered, *S.* ×*Readii* would almost certainly have maroon flowers, possibly with a little yellow-green about the edges or inner surfaces of the petals (from the *S. rubra* parent; see *S.* ×*Chelsoni*). Even without flowers, the parentage is unmistakable. The tubular pitchers and delicate appearance, as well as the apiculate hood of the hybrid, are definitely *S. rubra* features (Plate 2, fig. 1); and, as mentioned in other crosses involving *S. Drummondii*, the white coloration about the hood and orifice could be due only to this species.

11. *S. Drummondii* × *Sledgei*

Sarracenia ×areolata (Macfar.) Bell, stat. nov.

S. areolata Macfar. in Bailey Stand. Cycl. Hort. **6**: 3081. 1917.

Plants intermediate between parents. Summer leaves erect, elongate, narrowly trumpet-shaped, 3.2–7.5 dm. tall (usually 4–6.5 dm.), narrowly and evenly tapered from base to orifice, green, marked or shaded with red, areoles around orifice pale gray-green, or white, adaxial wing linear, very narrow; winter or late summer leaves ensiform or with recurved tips, 2.5–3.6 dm. tall, green, persistent; hood of summer leaves suberect, reniform to cordate (obtuse to apiculate) or suborbicular, 3.1–7.7 cm. broad (usually 4–6 cm.) narrowed and reflexed at the base, white or pale gray-green, rarely red, marked with more or less heavy red-brown or maroon veins, hood margins slightly revolute and undulate, adaxial surface hispid, the white hairs pointing toward the orifice, abaxial surface pubescent. Scape may or may not equal the length of the summer leaves. Flowers large, to 10 cm. or more across; sepals ovate to oblong, obtuse, brown-green or maroon on outer surface, greenish within; petals maroon outer surface, red-yellow within, or pinkish with yellow margins.

Type locality: Theodore, Mobile Co., Alabama. 1909.

Possible range: In association with parent species in the limited range of coastal southwestern Alabama and southeastern Mississippi.

Representative specimens: ALABAMA. Bay Minette, Baldwin Co., *Macfarlane* (PENN 38479); Theodore, Mobile Co., *Macfarlane* (CNC: lectotype), *Bell 544* (CNC); Deer Park, Washington Co., *Reade* (PENN 57863).

Despite its limited potential range, this is probably the most common hybrid *Sarracenia*. The two parent species hybridize freely in the areas around Mobile Bay and the offspring flower freely and backcross readily. The individuals resulting from these backcrosses are not as colorful as the original hybrid plants and they tend to make the lines of differentiation between the parent species and *S.* ×*areolata* rather obscure in some localities.

Sarracenia ×*areolata* is very similar to *S.* ×*Mooreana*, but when several specimens of each are placed together for comparison, the more slender pitchers and generally smaller hoods of *S.* ×*areolata* are noticeable (see Plate 7, figs. 1–2). Hood-shape in this hybrid is quite variable, as shown by one of Macfarlane's collections (PENN 38481); here, leaves with reniform hoods, and also leaves

with cordate-apiculate hoods, are found on the same plant. Coloration also varies, the lighter or darker forms of *S. Drummondii* influencing the hybrid offspring to a marked degree.

The red band across the constricted base of the hood, that is so prominent in most plants of *S.* ×*Mooreana*, is found only in a few of the plants of *S.* ×*areolata;* and these are all from the vicinity of Bay Minette, Baldwin Co., Alabama, *Macfarlane* (PENN 38478 and 38482). Some of these plants were labeled "*Sarracenia Drummondii* × *S. flava*" by Macfarlane, and although *S. flava, per se*, is not known from that area today, this brings up the possibility that these plants *are* actually hybrids between *S. Drummondii* and *S. flava*, which in all probability grew in this area at one time. The size of the leaves of some of these Bay Minette hybrids, *Macfarlane* (PENN 38480), would also tend to support this theory. Since the parentage of these "atypical" hybrids cannot be positively demonstrated without living plants, it is probably best to note their variations and not assign them definitely to either *S.* ×*Mooreana* or *S.* ×*areolata.*

12. *S. flava* × *minor*

Sarracenia ×**Harperi** hybr. nov.

S. crispata Hort. Bull ex André in Macfar. Pflanzenreich **4**: pt. 110: 36. 1908; *non* Bull ex André Illust. Hort. **27**: 87. 1880.

Sarracenia ×Harperi, hybrida naturalis inter S. flavam et S. minorem nova foliis erectis tubulatis e basi ad foramen versus diametro gradatim lateque accrescentibus luteo-viridibus rubro maculatis 2.2–4.5 dm. altis ala adaxiali lineari angusta infra medium latissima cucullo suberecto cordato basi angustato luteo-viridi colori rubiginoso suffuso vel venoso superficie adaxiali hispidulosa floribus mihi ignotis.

Plants intermediate between parents. Leaves erect, 2.2–4.5 dm. tall, widely tapered from base to orifice, yellowish-green only slightly marked with red around the upper portions, adaxial wing linear, to 1 cm. broad, widest below the middle; some leaves with reduced pitchers; hood suberect, cordate, 3–8 cm. broad, narrowed at the base, yellow-green, shaded or veined with maroon or red-brown, adaxial surface hispidulose, the short (less than 0.5 mm.) white hairs pointing toward the orifice, abaxial surface pubescent. Flowers unknown.

Type locality: Sandy bog in pine-barrens near Bloys, Bulloch Co., Georgia. 1901.

Possible range: In association with parent species in extreme southeastern North Carolina, more extensively in the coastal half of South Carolina and southward through southeastern Georgia into northern Florida.

Specimens examined: GEORGIA. Bloys, Bulloch Co., *Harper 855* (US 400381: type); Douglas, Coffee Co., *Harper 1437* (NY).

The hybrid nature of this plant was correctly ascertained in 1901 by Harper (1903), although at the time he had collected only one leaf. He located and photographed the hybrid at other localities the following year. These photographs were not only published by Harper (1905, 1918), but copies were at-

tached to the herbarium specimens of the plants and aid greatly in their characterization and identification. Harper (1918) also reports that this natural hybrid was collected near Summerville, Dorchester Co., South Carolina, by Macfarlane. In view of the extensive common range of the parent species and Harper's reference (1904) to "several specimens within a few feet of each other" (which could be either several individuals or an old clone) it is surprising that *S.* ×*Harperi* has not been reported more recently.

Flowers, which would be of little help in the identification of this hybrid, but would greatly aid in definitely separating it from the offspring of *S. minor* × *S. rubra*, have not been reported; and Harper's attempts to get blooms under cultivation failed. So he considered it a sterile hybrid.

The leaf characters of *S.* ×*Harperi* are intermediate between those of the parent species, as to size and shape (Plate 6, fig. 3), but the translucent areoles so conspicuous on the abaxial side of the leaves of *S. minor* are apparently totally absent in the hybrid. They cannot be detected in herbarium material, and Harper's various references to this natural hybrid omit any mention of this character. Even so, there can be no doubt as to the parentage of the plants in question, since the size and taper of the leaves and the slightly revolute hood margins (as shown in Harper's photographs) are *S. flava* characters, while the shape and "arch" of the hood are reminiscent only of *S. minor*. The extremely short hairs of the adaxial surface of the hood are found only in *S.* ×*Harperi* and *S.* ×*Rehderi*.

According to Andre (1880), Mr. M. W. Bull, an English nurseryman who imported *Sarracenia* plants for the trade, applied the name *S. crispata* to plants that he considered to be natural hybrids between *S. flava* and *S. rubra*. From the illustration and the description, Bull's plants were obviously not such hybrids, but probably plants of *S. Sledgei*. How Macfarlane (1908) ever came to consider *S. crispata* to be a hybrid between *S. flava* and *S. minor* is a mystery, especially since he guessed that it might be *S. Sledgei*. The cross between these two yellow-flowered species, *S. flava* and *S. minor*, does not appear to have been made by horticulturists. Since the hybrid thus lacks a name, it seems appropriate to designate it for R. M. Harper, who first collected it, and whose extensive observations and critical publications on the genus *Sarracenia* have been of value and interest to botanist and layman alike.

13. *S. minor* × *psittacina*

Sarracenia ×**formosa** Veitch ex Masters, Gard. Chron. ser. 2, **16:** 41. 1881.

S. formosa × Veitch ex Masters, Gard. Chron. ser. 2, **16:** 41. 1881.
S. Maddisoniana Nichols. Dict. Gard. **3:** 364. 1887.
S. decora Hort. ex Masters, Gard. Chron. ser. 3, **6:** 250. 1889.
S. Maddisonia Nichols. Dict. Pratq. D'Hort. **4:** 641. 1896.

Plants intermediate between parents. Pitchered leaves decumbent to suberect, 0.8–2.5 dm. long, tapered from base to orifice, yellow-green, with red reticulation and white areoles toward base of hood, adaxial wing broad (to 2 cm.), slightly undulate, widest at or slightly below the middle; some leaves with reduced pitchers and wider wings; hood somewhat globose, arching com-

pletely over the oblique orifice, yellow-green, lightly veined with red and lightly mottled with opaque white areoles toward the base, adaxial surface hispid, abaxial surface glabrous or lightly pubescent. Scape longer than leaves. Floral characters unknown.

Type locality: Moist pine-barrens near Fitzgerald, Irwin Co., Georgia. 1904.

Possible range: In association with parent species in the southern half of Georgia and portions of northern Florida west to Tallahassee.

Specimens examined: GEORGIA. Fitzgerald, Irwin Co., *Harper 2211* (NY: neotype; US 511228).

Harper (1906) also mentions this hybrid from Colquitt, Coffee and Wilcox counties, Georgia. Macfarlane (1908) mentions receiving a plant of this hybrid from "S. E. Georgia," but it does not seem to have been collected again (or collected elsewhere) in the last forty-five years.

This hybrid was well known as a horticultural plant in England, but no descriptions of its flowers are to be found. Harper had no information about them, having observed only old capsules on some of the various plants he reported. The leaves of *S.* ×*formosa* show the definite opaque markings of *S. minor* and the "intermediate" cucullate-globose hood (Plate 6, figs. 1–2) of the two parents. The hood is not only rather awkward to describe, but folds when pressed, so that its natural form is difficult to interpret. (Compare figs. mentioned above.)

14. *S. minor* × *rubra*

Sarracenia ×Rehderi hybr. nov.

S. minor × *rubra* Wherry, Jour. Wash. Acad. Sci. **19**: 383. 1929.

Sarracenia ×*Rehderi* hybrida naturalis inter S. minorem et S. rubram nova foliis erectis tubulatis e basi ad foramen versus diametro gradatim accrescentibus luteo-viridibus ad apicem versus rubromaculatis areolis albis paucis parvisque ornatis 1.5–2.5 dm. altis ala adaxiali lineari infra medium latissima cucullo suberecto cordato apiculato ad basem versus leviter constricto luteo-viridi venis rubris reticulato 2–4.5 cm. lato superficie adaxiali hispida vel hispidula floribus suaveolentibus sepalis leviter recurvatis rubiginosis petalis obovatis rubiginosis luteisque.

Plants intermediate between parents. Leaves erect, 1.5–2.5 dm. tall, gradually tapering from base to orifice, yellow-green, upper half reticulated with maroon veins, a few reduced, white areoles scattered along abaxial surface near the base of the hood, adaxial wing essentially linear, 1 cm. or less in width, widest below the middle; some leaves with reduced pitchers and wider wing; hood suberect, cordate, apiculate, 2–4.5 cm. broad, slightly narrowed toward base, yellow-green shaded or veined with maroon or red-brown, adaxial surface hispid to hispidulose, the white hairs pointing toward the orifice, adaxial surface finely pubescent. Scape much taller than leaves. Flowers fragrant; sepals ovate, more or less recurved, maroon, tinged or edged with yellow; petals obovate, maroon edged with yellow outside, red-yellow inside.

Type locality: Damp savannahs about 12 miles southwest of Shallotte, Brunswick Co., North Carolina. 1952.

Possible range: In association with parent species in southeastern North Carolina, the coastal half of South Carolina and southward through lower Georgia into northern Florida as far west as Tallahassee.

Specimen examined: NORTH CAROLINA. Shallottee, Brunswick Co., *Rehder* (CNC: type).

This colorful natural hybrid was first collected by Wherry (1929) in Candler Co., Georgia in 1923. The one specimen of the "Candler Pitcherplant," as it was called, was grown for six or more years at the United States Department of Agriculture greenhouse, but no herbarium material of this original collection has been located. The recent discovery of some of these plants in North Carolina is another result of Mr. Henry Rehder's careful observations (See *S.* ×*Swaniana*), and this hybrid is named in his honor.

Harper (1903), speaking of the hybrid *S. flava* × *minor*, says (page 333), "Where I collected it both of the supposed parents (as well as *S. rubra*, which, however, could have had nothing to do with it) were growing in the immediate vicinity." Despite this definite statement it seems quite possible that one plant of the collection, *Harper 1437*, might be *S. minor* × *rubra*, and this plant (US 431741) is not cited under *S.* ×*Harperi* for that reason. From the rhizome, it is obviously a mature plant; yet it is much smaller than the other hybrids supposedly of the same parentage. This is one of the few cases where flower color would definitely indicate which of the two possible hybrids we are dealing with. Unfortunately, none of Harper's specimens of this collection has flowers. The hispidulose character of the hood is similar in both the hybrids mentioned above. So this cannot aid in their separation.

The shape and markings of the leaf of *S.* ×*Rehderi* (Plate 5, figs. 1b and 2b) show the definite effect of *S. minor* in the cross; and the red coloration of the petals, slight reflexing of the sepals, and the sweet scent of the flower are due to the *S. rubra* parent.

15. *S. flava* × *rubra*

Sarracenia ×**Popei** Masters, Gard. Chron. ser. 2, **16:** 40. 1881.

S. Popei × Masters, Gard. Chron. ser. 2, **16:** 40. 1881.
S. crispata Bull ex Andre, Illust. Hortic. **27:** 87. 1880; *non* Hort. Bull in Macfar. Pflanzenreich **4:** pt. 110: 36. 1908.

Plants intermediate between parents. Leaves erect, ascending, 4–6 dm. tall, narrowly tapered from base to orifice, light yellow-green, upper portions marked or shaded with maroon, adaxial wing narrow, linear, or only slightly undulate, widest near the middle; hood suberect or horizontal, 3.5–5.2 cm. broad, ovate, acuminate, green, marked with dark red, adaxial surface puberulent, abaxial surface essentially glabrous. Scape equalling the height of the leaves. Flowers large, 10 cm. or more in diameter; sepals ovate-oblong, the margins more or less strongly reflexed, maroon or greenish-red on outer surface, yellow-green inside; petals oblong-obovate, maroon margined with yellow outside, yellow-pink inside.

Type locality: Carthage, Moore Co., North Carolina. 1926.

Possible range: In association with parent species from southeastern North Carolina southward through the coastal half of South Carolina and southern Georgia into northern Florida and westward through southern Alabama to the vicinity of Baldwin County, Alabama.

Specimen examined: NORTH CAROLINA. Carthage, Moore Co., *Harriot* (PH 815520: neotype).

The extensive coincident ranges of the two parent species of *S.* ×*Popei* do not seem to have had a very favorable influence on the occurrence of this natural hybrid, for only the single specimen mentioned above has been reported.

The unusually long, apiculate hood (Plate 4, fig. 2a) is not as pronounced in the type specimen, but the more or less reflexed sepals, the obovate petals, and the maroon coloration of the flower as a whole attest to the presence of *S. rubra* genes in the hybrid. The leaf and flower size and the yellow shadings in the flower are the main features inherited from *S. flava*. Notes on scent were not found, but should be interesting, because *S. rubra* has a very sweet scent and *S. flava* a rather strong, unpleasant, musty scent.

16. *S. psittacina* × *rubra*

Unreported either as a natural or as an artificial hybrid.

Possible range: In association with parent species in southern Georgia and northern Florida west through southern Alabama into southeastern coastal Mississippi.

17. *S. rubra* × *Sledgei*

Unreported either as a natural or as an artificial hybrid.

Possible range: In association with parent species in limited areas of southwestern Alabama and southeastern Mississippi near the Gulf coast.

Macfarlane labeled two sheets of material collected by Reade in 1913 as "Sarracenia? rubra × Sledgei." The locality was uncertain, and the material is very doubtful, despite Macfarlane's note on one voucher, "a small yellow-flowered form like rubra." These two sheets (PENN 57853 and 57864) should probably be considered as *S. rubra*, even though one leaf (the longest) on the first sheet is rather atypical for this species, especially as to hood shape. *S. rubra* × *Sledgei*, when found, should have much the appearance of *S.* ×*Popei*, perhaps only being a little smaller.

18. *S. flava* × *psittacina*

Unreported either as a natural or as an artificial hybrid.

Possible range: In association with parent species in southern Georgia and northern Florida and westward into southern Alabama.

19. *S. psittacina* × *Sledgei*

Unreported either as a natural or as an artificial hybrid.

Possible range: In association with parent species in the limited range of southwestern Alabama (generally on a line west of Mobile) into southeastern Mississippi near the Gulf coast.

DISPOSITION OF OTHER NAMES

No attempt has been made at the present to treat taxonomically the few reported natural *Sarracenia* backcrosses; therefore the various published horticultural names which would apply to such plants, as well as the names for the numerous compound *Sarracenia* hybrids (all of which have been made under cultivation), are included in the following list. Also included are quite a number of horticultural names which must be considered as synonyms of some of the accepted specific names in this genus. A significant percentage of the forty or more names considered below were not properly indexed, if indexed at all, in their original publications. These names were turned up through cross-checking references and by the rather unscientific but rewarding practice of just leafing through likely horticultural volumes. Without proper indexing, these names have not been noticed by Botanists in general, and have been excluded from proper taxonomic treatment. Also, without proper indexing, there is no way to be sure that the following list is complete, despite the effort to make it so.

Disposition of other published names in the genus *Sarracenia* not previously treated in this paper or the earlier one (Bell 1949) is made as follows:

S. alba Hort. ex Masters, Gard. Chron. ser. 2, **18:** 715. 1882. = ?*S. Drummondii* Croom.

S. Arkwrightii Hort. Bruce, ex Gard. Chron. ser. 3, **53:** 357. 1913. = ?*S.* ×*Mooreana.*

S. Ashbridgei Hort. Bruce ex Roy. Hort. Soc. Jour. **42:** clix. 1916. Nomen nudum.

S. atropurpurea Hort. ex S. G. Garden **28:** 219, 1885. = *S. flava* L.

S. atrosanguinea Nichols. Dict. Gard. Suppl. p. 654. 1901. = *S. purpurea* L. (according to Nicholson, but *atrosanguinea* was previously a var. of *S. flava.* See Masters, Gard. Chron. ser. 2, **16:** 12. 1881.)

S. aurea Sartwell, in Macfar. Das Pflanzenreich **4:** pt. 110. 1908. Manuscript name for *S. purpurea* f. *heterophylla.*

S. Buchanii Hort. Williams ex Roy. Hort. Soc. Jour. 1885. Nomen nudum.

S. Catesbaei Ell. of Macfarlane Contr. Bot. Lab. Univ. Penn. **2:** 426–434. 1904. = *S. Sledgei* Macf.

S. Claytonii Hort. Clayton ex Roy. Hort. Soc. Jour. **12:** cxv. 1890. Nomen nudum.

S. Cookiana Hort. ex Macf. Pflanzenreich **4:** pt. 110: 38. 1908. Nomen nudum.

S. crispata Bull ex Andre Illust. Hortic. **27:** 87. 1880. = ?*S. Sledgei* Macfar.

S. hyb. Diesneriana Hefka, in Oestr. Gart. Zeit. **8:** 43. 1913. = *S.* ×*Courtii* × *S. flava.*

S. Dormeri Hort. Veitch ex Roy. Hort. Soc. Jour. **26:** clxxix. 1901. = ?*S.* ×*Catesbaei.*

S. Drummondii alba Hort. ex Nicholson, Dict. Gard. **3:** 361. 1887. = *S. Drummondii* Croom.

S. Drummondii major Hort. ex Roy. Hort. Soc. Jour. **19:** clxxxvi. = *S. Drummondii* Croom.

S. Drummondii rubra Hort. ex Nicholson Dict. Gard. **3:** 361. 1887. = *S. Drummondii* Croom

S. Drummondii var. *undulata* Bailey, Cycl. Amer. Hort. p. 1616. 1902. = *S. Drummondii* Croom.

S. erythropus × Hort. ex Gard. Chron. ser. 2, **18:** 713. 1882. = *S. flava* L.

S. Farnhami × Hort. Farnham ex Roy Hort. Soc. Jour. **15:** cxx. 1892. Nomen nudum. (?*S. Drummondii* × *rubra* Hort. ex Macfar. Pflanzenreich **4:** pt. 110: 38. 1908.)

S. Fildesi Hort. Williams ex Masters, Gard. Chron. ser. 2, **16:** 11. 1881. = ?*S. Sledgei* Macfar.

S. flava Catesbaei Nichols. Dict. Gard. **3:** 364. 1887. = *S. Sledgei* Macfar.

S. flava var. *Catesbaei* (Elliott) Masters, Gard. Chron. ser. 2, **16:** 11. 1881. = *S. Sledgei* Macfar.

S. flava var. *erythropus* Hort. Bull ex Masters, Gard. Chron. ser. 2, **16:** 11. 1881. = *S. flava* L.
S. flava gigantea Hort. Bruce ex Roy. Hort. Soc. Jour. **31:** cxxxi. 1906. = *S. flava* L.
S. flava picta Nichols. Dict. Gard. **3:** 364. 1887. = *S. Sledgei* Macfar.
S. flava rubra Hort. Manda, in Roy. Hort. Soc. Jour. **15:** 135–143. 1892. = *S. flava* L.
Sarazina grandiflora Rafinesque, Aut. Botanikon p. 33. 1840. ex Boivin, Canad. Field-Nat. **65:** 21. 1951. Nomen alternativum.
S. heterophylla Ell. ex Steudel in Boivin, Canadian Field-Naturalist **65:** 22. 1951. = *S. flava* L.
S. illustrata Nichols. Dict. Gard. **3:** 364. 1887. = *S. Sledgei* × *S.* ×*Catesbaei*.
S. hyb. Laschkei Hefka in Oestr. Gart. Zeit. **8:** 43. 1913. = *S.* ×*Courtii* × *S.* ×*Mooreana*.
S. macrophylla Hort. ex Gentil, Pl. Cult. Serres Jard. Bot. Brux. 173. 1907. Nomen nudum.
S. melanorhoda Hort. Veitch ex Gard. Chron. ser. 2, **16:** 40. 1881. = *S.* ×*Catesbaei* × *S. purpurea*.
S. Mesoniana Hort. ex Gard. Chron. ser. 3, **6:** 81. 1889. Nomen nudum.
S. mexicana Hort. Moore ex Masters Gard. Chron. ser. 2, **16:** 11. 1881. = *S. Drummondii* Croom.
S. nigra Walt ex Higley, Bull. Chicago Acad. of Sciences **1:** 42. 1885. = *S. rubra* Walt.
S. nutans D. Dietr. Syn. p. 260. 1843. ex Macfar. Pflanzenreich **4**, pt. 110: 24. 1908. = *Heliamphora nutans* Benth.
S. Patersoniana Hort. ex Manda Roy. Hort. Soc. Jour. **15:** 142. 1893. = ?*S.* X*Mitchelliana* (Nicholson) Bell.
S. psittacina var. *minor* Hook. Jour. Bot. London **1:** 190. 1834. = *S. psittacina*.
S. purpurea var. *ripicola* Boivin, Canad. Field-Nat. **65:** 21. 1951. = *S. purpurea* L.
S. purpurea var. *stolonifera* Macfar. et Steckb., Kew Bull. Misc. Inform. **4.** 1933. = *S. purpurea* L.
S. purpurea L. var. *terrae-novae* Pylaie f. *heterophylla* Boivin, Canadian Field-Naturalist **65:** 22. 1951. = *S. purpurea* f. *heterophylla* (Eaton) Fernald.
S. rubra Walt. of Steudel (not Walt. and other authors), Nomen. Bot. **2:** 733. 1821. = *S. psittacina* Michx.
S. Sanderae Hort. Sanders ex Roy. Hort. Soc. Jour. **20:** cci. 1896. = *S. Drummondii* × *S.* ×*Cookiana*.
S. Sanderiana Hort. Sanders ex Roy. Hort. Soc. Jour. **20:** cxxxiii. 1896. = *S. Drummondii* × *S.* ×*Readii*.
S. hyb. Schoenbrunnensis Hefka, in Oestr. Gart. Zeit. **8:** 43. 1913. = *S.* ×*Courtii* × *S.* ×*Cookiana*.
S. terrae-novae Pylaie, Mem. Soc. Linn. Paris **6:** 389. 1827. ex Wherry Bartonia **15:** 1–6. 1933. = *S. purpurea* L.
S. hyb. Umlauftiana Hefta, in Oestr. Gart. Zeit. **8:** 43. 1913. = *S.* ×*Courtii* × *S.* ×*Wrigleyana*.
S. hyb. Vetteriana Hefka, in Oestr. Gart. Zeit. **8:** 43. 1913. = *S. illustrata* × *S.* ×*Catesbaei*.
S. viridis Hort. ex The Garden **24:** 447. 1883. = *S. purpurea* L.
S. vittata maculata Nichols. Dict. Gard. Suppl. 1901. = *S. purpurea* × *S.* ×*Chelsoni*.
S. hyb. Vogeliana Hefka in Oestr. Gart. Zeit. **8:** 42. = *S.* ×*Courtii* × *S.* ×*Catesbaei*.
S. Willisii Hort. Veitch ex Roy. Hort. Soc. Jour. **17:** lxxiv. 1894. = *S.* ×*Courtii* × *S.* ×*melanorhoda*.
S. × *Willmottae* Hort. Bruce ex Roy. Hort. Soc. Jour. **36:** cxxviii. 1910. = *S.* ×*Catesbaei* × *S. purpurea*.

SUMMARY

In order to make future identification of hybrids in the genus *Sarracenia* more accurate, and the nomenclature of these plants more stable, the rather extensive horticultural, as well as botanical, literature was reviewed. From this review, and from a study of herbarium material—in the light of field notes and

greenhouse data on *Sarracenia* hybrids—descriptions were drawn for all reported natural hybrids in this genus. Figures and photographs are also presented, to aid in proper identification of these plants. The numerous varietal and horticultural names not treated under the hybrids considered here, or under the various species of *Sarracenia* dealt with earlier (Bell 1949), are listed with their references, and assigned to appropriate categories.

One new natural hybrid is reported and several previously reported but undescribed natural *Sarracenia* hybrids are given proper descriptive treatment.

ACKNOWLEDGMENTS

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EXPLANATION OF PLATES

PLATE 2

1. *S.* ×*Readii* (*S. Drummondii* × *rubra*). From type specimen, *Reade* (PENN 57859). ×½.

2. *S.* ×*Mitchelliana* (*S. Drummondii* × *purpurea*). *Bell* 2-19 in Botany Dept. greenhouse, Univ. of North Carolina, 1949. Plant from Baldwin Co., Ala. *Bell 548* (CNC). ×½.

3. *S.* ×*Chelsoni* (*S. purpurea* × *rubra* f. *Jonesii*). From Etowah, Henderson Co., N. C., 1933. *Clements* (CNC). ×½. Compare with Plate 4, fig. 1 and Plate 7, fig. 4.

PLATE 3

1. Adaxial view of fresh leaves, slightly under ×½.

a. *S. minor*

b. *S.* ×*Swaniana*

c. *S. purpurea*

2. Side view of same leaves, which were sent to me by Mr. Rehder from collections made in the vicinity of Wilmington, Brunswick Co., N. C. Leaves pressed, *Bell 1124, 1122, 1126* (CNC).

PLATE 4

1. *S.* ×*Chelsoni* (*S. purpurea* × *rubra*). Plant from which type specimen was taken. ×½.

2. Photograph of Plate 566 *The Garden* **30**: 366. 1886.

a. (top leaf and flower) *S.* ×*Popei* (*S. flava* × *rubra*).

b. (lower leaf and flower) *S.* ×*Mooreana* (*S. Drummondii* × *flava*).

PLATE 5

1. Abaxial view of fresh leaves, slightly under ×½.
a. *S. minor*
b. *S.* ×*Rehderi*
c. *S. rubra*
2. Side view of same leaves, which were sent to me by Mr. Rehder from collections made in the vicinity of Wilmington, Brunswick Co., N. C. Leaves pressed, *Bell 1124, 1121, 1125* (CNC).

PLATE 6

1. *S.* ×*formosa* (*S. minor* × *psittacina*); photograph of portion of fig. 419 *Dictionary of Gardening* **3**: 365. 1887. Compare with fig. 2.
2. *S.* ×*formosa* (*S. minor* × *psittacina*); photograph of type specimen, *Harper 2211* (NY), from Irwin Co., Georgia. ×½.
3. *S.* ×*Harperi* (*S. flava* × *minor*); photograph of type specimen, *Harper 855* (US 400381), from Bulloch Co., Georgia. ×½.
4. *S.* ×*Courtii* (*S. psittacina* × *purpurea*); photograph of type specimen, *Harrison* (US 2997), from cultivation. Slightly over ×1.

PLATE 7

1. *S.* ×*areolata* (*S. Drummondii* × *Sledgei*); photograph of portion of collection, *Macfarlane* (PENN 49331), from Theodore, Mobile Co., Alabama. Slightly under ×½.
2. *S.* ×*Mooreana* (*S. Drummondii* × *flava*); photograph of pressed plant from Crestview, Florida. *Macfarlane* (PENN 51337). ×½.
3. Extremely large leaf of *S.* ×*Catesbaei* (*S. flava* × *purpurea*); from Ponce de Leon, Florida. *Macfarlane* (PENN 38474). Slightly less than ×½. (The hood is 13.7 cm. across at the widest point!)
4. Leaf of *S.* ×*Chelsoni* (*S. rubra* × *purpurea*); Redrawn from fig. 148A, *Gardeners' Chronicle* ser. 2, **15**: 821. 1881. Note reniform hood although *S. purpurea* is the pollen parent. ×½. Compare with Plate 2, fig. 3, and Plate 4, fig. 1.

PLATE 2

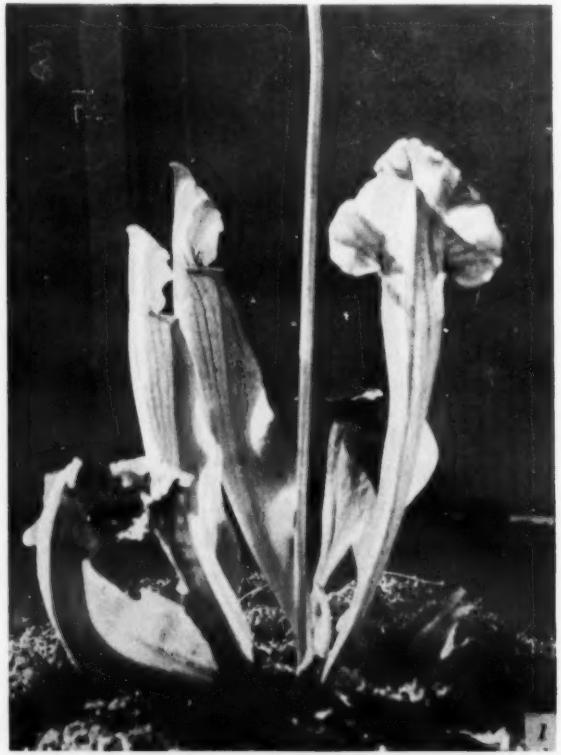
1

2

3

PLATE 3

PLATE 4



1

2

PLATE 5

PLATE 6

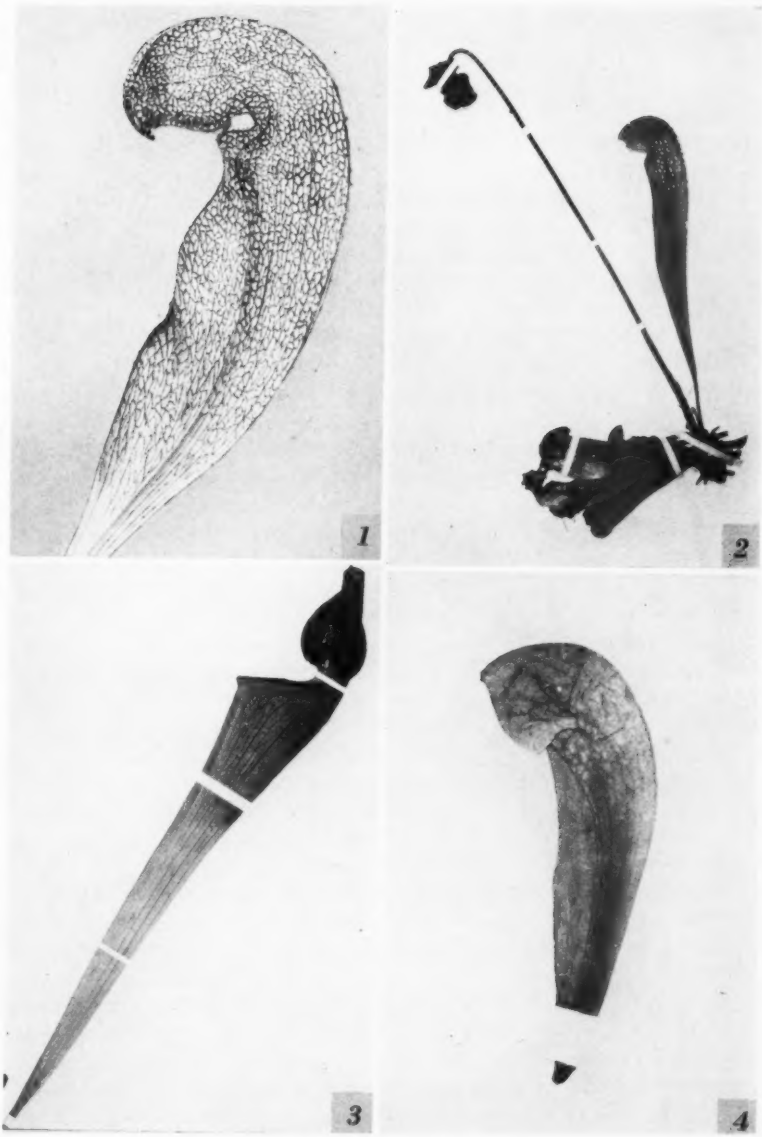
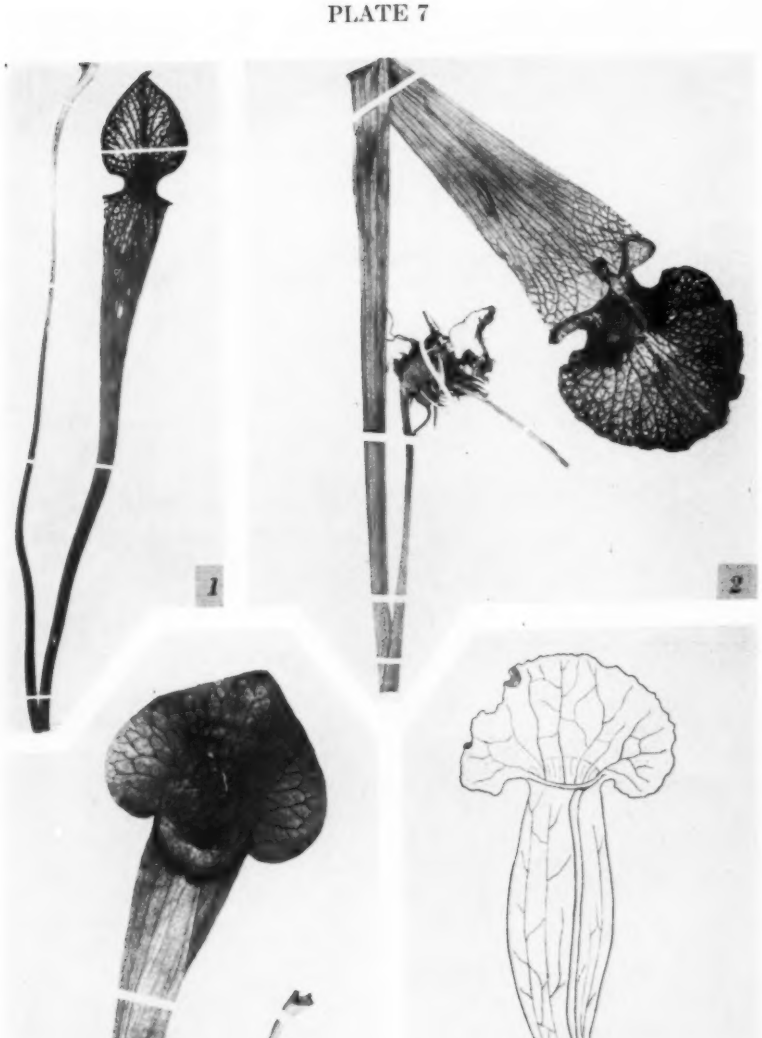


PLATE 7



AN ADDITION TO THE FERN FLORA OF NORTH CAROLINA

By Lewis E. Anderson[1] and Thomas T. Bannister[2]

Plate 8

In 1946, the Rev. A. Rufus Morgan, of Franklin, North Carolina, an enthusiastic and discerning student of local ferns, discovered *Asplenium monanthes* L. along the Whitewater River in Oconee County, South Carolina (Blomquist, 1948). This is a widely distributed, essentially tropical fern which is known from Africa, several islands of the Atlantic, Jamaica, and the Americas, from Chile through Mexico to Arizona (Maxon, 1913). The remarkable discovery of this fern in South Carolina thus added a disjunct station some 1,500 miles from its nearest previously known locality in Arizona. It was the good fortune of the senior author, along with Dr. Henry J. Oosting and Mr. Robert L. Wilbur, to accompany Mr. Morgan to the Whitewater River locality in the summer of 1947, and observe *A. monanthes* in the field. A number of colonies of this interesting fern have since been found along the Whitewater a mile or so above and below its junction with the Thompson River. Since the headwaters of both the Thompson and the Whitewater are in Jackson County and Transylvania County, North Carolina, it seemed reasonable to suppose that *A. monanthes* might be found in North Carolina. Consequently, in the summer of 1949, while exploring this general area for bryophytes, a rather careful search for the fern was made.

The Whitewater River originates with the confluence of several small mountain streams with headwaters along Chattooga Ridge and Hogback Mt., on the southeastern Blue Ridge escarpment. For the first seven miles of its course, the Whitewater winds through a mildly rugged terrain of broken hills and long trailing spurs and ridges, with a gradient, over this distance, of about 400 feet. At this point, close to the North Carolina-South Carolina boundary, it makes a spectacular descent into the Piedmont, plunging from 2,800 feet to 800 feet in less than four miles. This torrential descent has produced a deep, narrow gorge with a vertical depth of from 300 to 400 feet in places, and a width of less than 1,000 feet. Two large and handsome waterfalls, one in each state, account for much of the rapid drop. The first, called the Upper Falls, is in Jackson-Transylvania Counties, North Carolina, and, altogether, accounts for a drop of approximately 460 feet. The second, called the Lower Falls, is in Oconee County, South Carolina, and, including a prolonged series of steep cascades, is responsible for a descent of about 1,200 feet.

The aforementioned area in which *A. monanthes* was reported is directly below the Lower Falls, extending several miles down stream, almost to the village of Jocassee, situated at the mouth of the gorge. The altitude is approximately 800 feet. The fern is not abundant for less than a dozen colonies have

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been observed in the entire area. The southwestern side of the gorge originally supported a dense, rich forest of hemlock and mixed hardwood species including tulip poplar, red maple and sugar maple, northern red oak, white oak, sweet gum, walnut, buckeye, basswood, cucumber tree and beech. This is indicative of Braun's (1935) Mixed Mesophytic Forest Association. In strong contrast, the opposite or northeastern side of the gorge, which is steeper and obviously drier, is occupied by pitch pine, shortleaf pine, virginia pine, chestnut oak, scarlet oak and occasional remnants of chestnut. As far as is now known, *A. monanthes* is confined to the Mixed Mesophytic habitat. Unfortunately, within the past three years, intensive and severe lumbering operations followed by fire have greatly altered the habitat in most of the area and many colonies of *A. monanthes* have disappeared.

Several attempts were made to locate *A. monanthes* in the area between the Lower and Upper Falls. It was immediately obvious that if the fern extended into this region of slightly increased altitude it was exceedingly rare. Finally, after several unsuccessful attempts in this area, a single colony of *A. monanthes* was found a short distance below the Upper Falls, in North Carolina. This single colony of about six plants was growing in a moss mat on a partially shaded boulder on the northeast side of the stream, about 300 feet below the junction of Corbin Creek. To minimize disturbance and preserve the colony, only a few fronds were removed for a specimen. When the colony was observed again in the summer of 1951, it was maintaining itself, although the number of plants had not increased.

Similar searches for *A. monanthes* were made in several of the other principal escarpment gorges, specifically those of the Chattooga, Thomson and Toxaway Rivers. No trace of it could be found in any of these regions. However, it is a fern that could easily be overlooked, for it is very similar in general appearance to our common ebony spleenwort, *A. platyneuron* (L.) Oakes. The fronds of *A. monanthes* are more slenderly acuminate and the lower pinnae show less tendency to decrease in size toward the base than in *A. platyneuron* (Plate 8). Furthermore, as Blomquist points out, the sori of the former are mostly confined to the lower margin of the pinnae and tend to be parallel to the lower margin.

Blomquist has already pointed out the parallel between the occurrence of *A. monanthes* and that of other tropical ferns that have been discovered in the same area, notably *Hymenophyllum tunbridgense* (L.) J. E. Smith, *Trichomanes Petersii* A. Gray (Taylor, 1938), and *T. Boschianum* Sturm (Coker, 1938). There are similar parallels among the bryophytes. Bartram (1951) has recently described *Glossadelphus Andersonii* from material collected by the senior author which was growing on a boulder with *Asplenium monanthes*. This moss belongs to a genus of wide tropical distribution, but is unknown elsewhere on the North American continent. Whitewater Gorge is also the type locality of *Plagiochila Sharpii* Blomq., which was also collected in the same general area. The closest relative of this hepatic is distributed in Mexico. An undescribed species of *Mnium*, with South American and Asiatic affinities, is commonly distributed

throughout the gorge. Numerous other examples of the floristic affinities of the Southern Appalachians and the tropics have been pointed out by Sharp (1939) and others. As so aptly stated by the eminent bryologist, Mr. E. B. Bartram (*in litt.*, December 6, 1951), these observations "... bring up conjectures of misty outline that will probably have to wait for a clearer definition."

SPECIMEN CITED: *Asplenium monanthes* L., rock crevice on granitic boulder, below the Upper Falls, Whitewater River, Transylvania County, North Carolina. *Anderson & Bannister 8759*, August 25, 1949 (Duke University Herbarium).

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PLATE 8

Asplenium monanthes L., on moss mat on boulder along Whitewater River, Transylvania County, North Carolina.

HEREDITARY ABNORMALITIES IN TOBACCO

By F. A. Wolf and D. G. Sharp*

Plates 9 to 11

During the past few years the writers have given consideration to certain disorders of tobacco that manifest themselves by structural abnormalities of the leaves. One of these has been designated "ruffles" and another "toad-skin." They are so dissimilar as to be regarded as distinct types but yet have in common the presence of enations i.e. wing-like, blade-like, or laminar excrescences arising from the midribs and secondary veins.

Such terms as teratological structures, monstrosities, deformities, malformations, anomalies, and teratomas have been applied to structural abnormalities of plant organs. Apparently abnormalities are not of unusual occurrence, as shown by the compendia of Masters (1869), Worsdell (1915, 1916), and Penzig (1921, 1922). In fact the entire first volume of the three volume series by Penzig contains little besides an alphabetical list, by authors, of references to studies on plant teratologies. Teratologic structures are stated to be incited by the action of quite distinct agencies such as viruses, bacteria, fungi, nematodes, mites, and insects, as well as by genetical and environmental factors. To date, however, the proximate causes of their formation remain quite unknown.

A survey of publications on excrescences or abnormal outgrowths on tobacco foliage indicates that several investigators have noted the incidence of abnormalities that are quite similar to, or are apparently identical with, ruffles and toad-skin. Moreover, it is established by our own studies and by the experimentation of certain others that these two abnormalities are not induced by an exogenous agency or factor but that they are genetic and, hence, heritable. The immediate purpose, therefore, is to present that results of our own studies of these two types of abnormalities and to collate them with pertinent investigations by others.

Historical†

Laminar excrescences on tobacco leaves appear to have been reported first a hundred years ago by Schimper (1851). The outgrowths which he described occurred on the veins and were goblet-like, hence, properly may be designated

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† The excellent genetical analysis of enation by Hitier (Hitier, Henri. Étude génetique des caractères "enation," et "catacorolle" chez *Nicotiana tabacum* L. 1950. Ann. Inst. Exp. Tabac de Bergerac **1**: 31–61) was unfortunately overlooked. We received Hitier's report through the courtesy of Dr. R. E. Clausen, University of California, Berkeley, Calif. In his hybridization studies he used Java, or Vuelto abajo, or P. 19 (*Brasiliensis*) x Enation 701, and also Enation 701 x Virginia, or *purpurea*, or Cabot. His analysis showed the genotypes of Enation 701 to be aa bb, of Java aa BB, of Cabot AA bb, and of the other varieties AA BB. Thus Enation 701, which appeared as a cross between Java and Cabot, is governed by two recessive factors one contributed by Java, the other by Cabot.

ascidia. Similar structures were described 40 years later by Buchenau (1891). Then, twenty years later, Lodewijks (1911) observed crest-like wings on the ribs of tobacco leaves. In 1914, Honing (1923) noted what he regarded as a mutation among Deli tobacco plants in fields in Sumatra and described this mutant as a new species, *Nicotiana deformis*, an appropriate name since the stems, leaves, and flowers were markedly deformed. When he hybridized *N. deformis* with normal Deli tobacco, segregation in the F_2 generation yielded a 1:2:1 ratio of normal, hybrid, and *deformis*, respectively. In a back cross of *deformis* with hybrid, as well as of hybrid with normal, each gave a ratio of 1:1 in the progeny.

In 1937, Lehman (1938) noted an abnormality among plants of a flue-cured tobacco variety growing near Raleigh, North Carolina, which he called "ruffles." The leaf margins of affected plants turned downwards with crimped accessory laminae or ruffles along the veins. In some instances the blade tissues were almost entirely lacking. The flowers were variously deformed. This syndrome apparently is like that studied by Honing (1923). It may be noted that Lehman concluded that the disorder was heritable.

Arisz (1927), using an East-Java variety of toabacco known as Kedoe, isolated certain lines having heritable abnormalities involving the flowers and leaves. One of these lines, having enations on the lower leaf surface, as photographically illustrated, (Arisz, 1927, *vide* p. 50) appears to be like the toad-skin disorder of the present account. An identical abnormality in an oriental tobacco variety, Aja Soluk, growing in Italy, was observed by Trotter (1939). He regarded it as a teratological mutation. Trotter compared this disorder with another one which had been under observation in the East Indies and in Africa for more than 20 years and which it resembled in certain respects. He correctly regarded the two as distinct. The virus nature of this other disorder had only recently become established from the work of Storey (1931) and Thung (1932). These investigators called the disease leaf curl, or the Dutch equivalent kroepoek, and found that the causal virus was insect-borne. Meanwhile, Moore (1934) observed a leaf-curl disease in South Africa which he found to be inherited and which, beyond doubt, is like the disease on oriental tobacco observed by Trotter. Unfortunately, Wolf (1935, *vide* p. 181), using Moore's illustration, confused this inherited disorder with the true virus-induced leaf curl or kroepoek disease.

Cuzin et al. (1947) and Grisquet (1948) studied a heritable monstrosity, which they called "enation 701", that appeared in the F_2 generation obtained by crossing Java-Isérè and Cabot. The leaves of affected plants were strongly incurved downward and bore dorsally along the midribs and secondary veins elongated, leaf-like excrescences.

Within the current year, Tirelli (1951) published an extensive account of the anatomy of enations occurring on a strain of Virginia Bright tobacco, a disorder first noted in Italy in 1919. In this account he divided enations into three basic types: 1) cord-like protuberances (net-like, enlarged veins); 2) crest-like, alar or laminar excrescences of varying lengths and widths, each having a free margin; and 3) cup-like structures (ascidia) occurring on the lower leaf surface, as sometimes appear in kroepoek.

OBSERVATIONS

Material.—Both of the abnormalities upon which the present study is based appeared spontaneously in field-grown plants. One of them arose within a Bashi Bagli variety of the Samsoun type of oriental tobacco and it has been found several times during the past 10 years. Another source of abnormal plants was seed supplied by Dr. W. D. Valleau, Agricultural Experiment Station, Lexington, Kentucky. This abnormality arose in a cross between the Bashi Bagli variety, mentioned above, and a Burley variety. These two foregoing sources provided specimens of the abnormality herein called toad-skin.

The abnormality called ruffles came from a flue-cured variety grown at Tifton, Georgia, the seed of which were supplied by Mr. J. M. Carr, Tobacco Experiment Station, Oxford, North Carolina. In 1943, the same disorder was observed by Dr. W. A. Jenkins, Chatham, Va., in a field of flue-cured tobacco in Virginia.

Description of toad-skin.—The first true leaf on young seedlings may be found to show this disorder. In other cases there is no evidence of abnormality until shortly after the seedlings have been transplanted into the field. As seen from the upper surface, the edges of the older leaves become enfolded or rolled upward more or less tightly to make two rolls (Plate 9, Fig. A). In extreme cases the leaf is also twisted on its long axis, sometimes making a complete revolution. As seen from the ventral surface, finger-like protrusions and wing-like enations varying from low ridges to prominent alar crests or flap-like proliferations appear along the midrib and secondary veins (Plate 9, Fig. B). These enations may sometimes bear secondary enations that may appear to have anastomosed with each other. In addition, the larger veins may be knotted and the smaller ones may appear crumpled or puckered, giving a warty or rugose surface; hence the name toad-skin. The stems and leaves of affected plants are a darker green than are those of normal plants. The flower parts are not deformed, nor is there any decrease in the number of capsules and seeds which such plants produce.

Description of ruffles.—In ruffles also the abnormality may be evident in small seedlings. The leaf margins roll downward and the leaves are downwardly arcuate (Plate 10). Instead of being sessile or having clasping bases, the leaves are long-petiolate. Eventually the blades of leaves near the top of the plant may be quite lacking, consisting of little besides midrib. In some cases the blade margins may anastomose to form pouches. Enations arise along the veins on the dorsal leaf surface. Such enations vary greatly in length and width. Even in any particular enation there may be great variation in its width, which condition, coupled with the folding of the enation on itself, produces the characteristic ruffle-like appearance. The flowers on ruffled plants exhibit a variety of deformities, including a splitting of the calyx lobes and corolla, and a doubling of the corolla (cata-corolla). The capsules are reduced both in size and in number and are not well-filled with seed.

Anatomy of enations.—Leaf tissues of toad-skin-affected plants were appropriately fixed, embedded in paraffin, sectioned, and stained*. On microscopic

* Mr. Wirt H. Wills prepared the sections used and we gratefully acknowledge his help.

examination these sections showed that the most outstanding feature was reversal of dorsiventrality in the enations (Fig. 1, A). In such inversions the palisade cells occupy the position where one might expect to find the spongy parenchyma (Fig. 1, D). The vascular tissues also were in reverse orientations (Fig. 1, C). This observation confirms the thorough-going anatomical studies by Tirelli

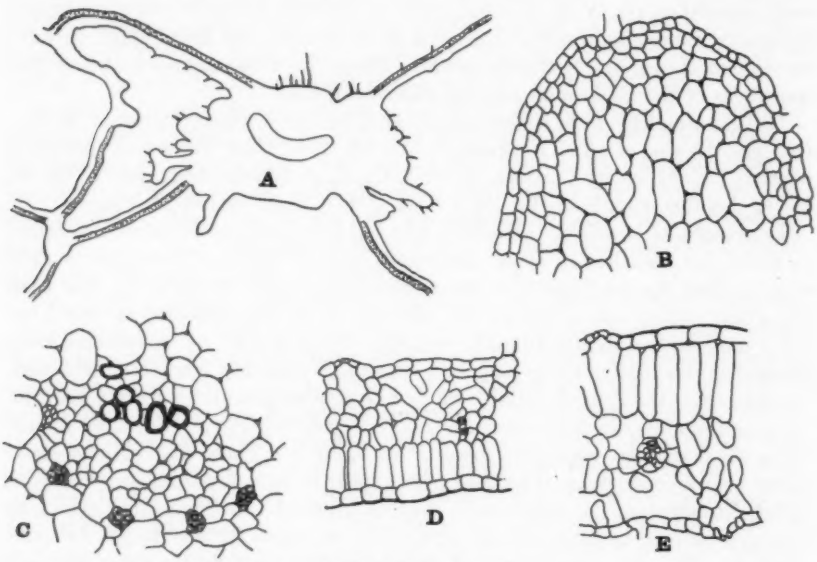

FIG. 1. Gross morphology of toad-skin-affected leaves.

A. Diagram of cross-section of midrib area. Stippled areas represent palisade parenchyma. The accessory bulging processes are young enations. In older enations dorsiventrality is reversed.

B. All cells of young enations are quite alike.

C. Cross-section of vascular bundle of vein in enation, with reversal in orientation of xylem and phloem.

D. Cross-section of blade of enation with palisade tissue on the downward side.

E. Arrangement of tissue in cross-section of a normal blade.

(1951). Tissue reversal of this sort in enations on tobacco had previously been noted by Kerling (1933), Lehman (1938), Grisquet (1948), and others.

The feature of secondary importance exhibited by these sections was that the enations appeared to arise from the cortical tissues of the veins in a fashion entirely like that which operates to produce the normal blade tissues. The enations arise as ridge-shaped protrusions consisting of parenchyma cells, all of which, except for the epidermis, are at first quite similar in appearance (Fig. 1, B). Moreover, the secondary veins possess cortical tissues having a potentiality for similar proliferation. The veins within the primary enations also possess the ability to proliferate to give rise to secondary proliferations or enations. In such

secondary enations there is reversal of dorsiventrality, bringing the tissue orientation back to that in normal leaves. In general, cell differentiation in enations appears to cease somewhat short of that which occurs in mature normal leaves (Fig. 1, E), thus leaving the tissues of enations somewhat juvenile (Fig. 1, D).

TRANSMISSIBILITY OF THE CAUSE OF ABNORMAL STRUCTURES

Three methods of approach were used in efforts to determine the nature of the agency which induces these anomalous structures on tobacco. One was the making of approach-grafts to test infectiousness; another was the examination of expressed sap with an electron microscope, to search for virus; and the other was hybridization trials, to test transmissibility.

Approach-grafts.—Two pairs of potted plants, each pair consisting of a normal plant and a toad-skin-affected one, were approach-grafted. This was done by cutting plane surfaces at the same height on the pair of plants and then binding them together at the cut surfaces, using grafting tape. After a few weeks, secure unions had been formed between the paired stems, and each member continued to grow. None of the new growth of the two normal members, either of the apical bud or of the axillary buds, subsequently exhibited any abnormality. Eventually flowers were produced on each member of the two pairs, and seed were matured. When seed from the normal ones were sown and the seedlings therefrom transplanted, all the resultant plants were found to be entirely normal in appearance, demonstrating a complete lack of transmission of a disease-producing agent by means of approach-grafting.

Examination of sap.—As has been indicated, certain of the symptoms exhibited by the abnormal plants herein studied are like those of the kroepoek disease. This fact suggested that the sap of abnormal plants might be found, on examination with the electron microscope, to contain virus. To accomplish this end, sap was expressed from the leaves of ruffled plants, of toad-skin-affected plants, and of normal plants. Each sap sample was clarified by filtration through celite on a Buchner funnel. A water pump served to produce the vacuum to aid filtration.

The cleared sap was then ultracentrifuged for 60 minutes at 47,000 G. The resulting pellet was next suspended in 0.06 M phosphate buffer, pH 7.2, to make the original volume. This suspension was spun at 3,000 G for 10 minutes and the pellet thus obtained was discarded. The supernatant fluid, however, was again ultracentrifuged at 47,000 G for 60 minutes. The precipitate obtained was then diluted, deposited on collodion, and chromium-shadow-cast at an angle of 15°.

Sap preparations of this kind contain approximately spherical particles of varying sizes ranging from about 75mμ in diameter to 15 or 20mμ; the larger ones give the impression of aggregates of smaller ones. Sap from plants affected with ruffles (Plate 11, A) shows such a range of polydisperse particulate material. Sap from leaves of the toad-skin type (Plate 11, B) shows somewhat smaller aggregates, but no characteristic differences between the two were observed. A sample of normal sap, purified through one cycle of ultracentrifugation instead of two, showed more of the uniformity ordinarily expected of plant viruses. Probably the particles (Plate 11, C) had been less aggregated by sedimentation into

pellets, with subsequent more incomplete resuspension, than were the other materials which were sedimented twice. These particles are 15–20mμ in diameter and there are insufficient differences between them and many of those of figures A and B (Plate 11) to warrant any statement about them other than that the so-called "macromolecules" of the sap of normal tobacco plants are regarded as similar to those in the sap of abnormal plants. Hence there is no evidence at hand in these representative photographs to indicate that toad-skin and ruffles are induced by a virus or viruses. Moreover, Loring, Osborn, and Wyckoff (1938), and Price and Wyckoff (1939), have reported that sap of normal tobacco yielded a component of S 20° = about 70×10^{-13}, which corresponds to particle diameters shown in ruffles-affected and in toad-skin-affected plants.

Hybridization tests.—A complete genetical analysis was not attempted, owing to limitations of time and of space in the greenhouse and field. The limited trials which were made, however, establish certain facts about the inheritance of each of the two types of abnormalities. When, for example, seed were obtained by self-pollination of certain of the plants affected with ruffles, and such seed were sown, all of the resultant plants exhibited deformities, varying among themselves only in degree of abnormality. It must be concluded, therefore, that in this instance a pure line of ruffled plants was concerned. Certain other ruffled plants, however, when self-pollinated produced seed which yielded plants some of which were ruffled and some of which were normal. The parents of such plants must have been hybrid, and among them abnormal was dominant over normal. If then one self-pollinated such normal progeny, the resultant seed were found to yield only normal plants. These trials therefore demonstrate that the abnormality called ruffles is heritable and that abnormal is dominant over normal.

Seed obtained by crossing a toad-skin-affected plant with a normal one yielded in the F_1 only normal-appearing plants. When F_1 individuals were self-pollinated, most of the progeny in the F_2 generation appeared to be normal but some of them exhibited the toad-skin abnormalities. Seed arising from self-pollination of these affected plants yielded a pure line of affected plants. In the case of this disorder, therefore, abnormal is recessive.

DISCUSSION

That structural abnormalities in animals and plants are heritable is supported by a vast body of evidence. By virtue of inheritance such abnormal organisms are appropriately regarded as mutations. As typifying the many instances of such inheritance among species other than tobacco may be cited the results of studies with coffee. Krug and Carvalho (1945) found that a single pair of genes controls the type of branching of coffee trees, the shape and size of the leaves, as well as the morphology of the flowers, fruits, and seeds. The F_1 of their hybrid of normal *Coffea arabica* with the variety *anomala* showed complete dominance of normal, but such hybrids, if self-pollinated, yielded in the F_2 generation a 1:2:1 ratio of normal, hybrid, and *anomala*, respectively.

Various investigators including Lodewijks (1911), Klebs (1916), Allard (1919) Trotter (1939), Cuzin, Fardy, and Schwartz (1941), Grisquet (1948), and Tirelli (1951) have contributed to our knowledge of mutations in *Nicotiana*. Certain

of them, notably Allard, Honing, and Arisz, made genetical analyses which showed that inheritance of each of the mutations studied follows a Mendelian pattern. It seems relatively unimportant whether the activity of a single gene or factor or of several genes or multiple factors are involved, to explain these mutations. Instead, it appears more germane to seek a proximate cause than to focus attention on genes as physical mechanisms of inheritance. Experiments involving crossing-over in plants leave little reason to doubt that positional rearrangement of genes occurs and that this phenomenon may be involved in mutations. Perhaps mutations, such as ruffles and toad-skin, should be expected to be of frequent occurrence in *Nicotiana tabacum* which is itself a natural hybrid. They should result most often from the hybridization of distinct tobacco types, and the greater the varietal differences of the parents the greater the likelihood of the occurrence of mutations.

It may be pointed out as having possible bearing on the problem of proximate causes of mutations such as the two types considered herein, that the features of kroepoek-diseased tobacco, as described by Kerling (1939), have many anatomical similarities in common with ruffles and toad-skin. The kroepoek virus may, therefore, be considered to modify the activity of genes in a somewhat similar proximate fashion as occurs without the mediation of a specific exogenous agent. As a consequence one is forced to accept the hypothesis that the proximate cause of malformations, whether heritable or induced by living pathogens, is mediated by genes and therefore is endogenous. Such proximate cause must therefore be sought among aberrations in enzyme activities, as postulated by Honing (1923), among unbalances in production of growth factors, as was proposed by Penzig (1921), or among responses to some specific chemical stimulus, as was suggested by Tirelli (1951).

One might, with good reason, question whether ruffles and toad-skin should be regarded as distinct types of abnormalities. The main reason for regarding each as an entity rests primarily upon the fact that the genetical evidence in hand shows that the former behaves as a dominant and the latter as a recessive.

SUMMARY

This account deals with two heritable structural abnormalities of cultivated tobacco that apparently are identical with disorders previously investigated in other regions. One is herein called ruffles and the other toad-skin.

Enations are developed on the leaves of affected plants in both disorders. They occur on the dorsal leaf surface in ruffles and on the ventral leaf surface in toad-skin.

Dorsiventrality is in reverse orientation in tissues of enations.

Evidence of the absence of an exogenous agent as a cause of these disorders rests upon failure of transmission, using approach-grafts, and upon failure to find virus in the expressed sap, using electron microscopy. Also, evidence from hybridization indicates that both disorders behave in Mendelian fashion, ruffles as dominant and toad-skin as recessive.

The proximate chemical environment which controls the activity of the genes which mediate these disorders remains unknown.

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EXPLANATION OF PLATES

PLATE 9

Leaves of toad-skin affected plants.

FIG. A. The leaf margins roll upward.

FIG. B. Enations are present on the lower leaf surface, on the midribs and secondary veins, and the interveinal tissues are crimped and drawn.

PLATE 10

Small ruffled plant. Note downward curvature of leaves, rolling-inward of the leaf margins, and enations on the upper leaf surface.

PLATE 11

Photographs of particles from sap from tobacco tissues, as shown by the electron microscope.

FIG. A. Ruffles sap—a two cycle ultracentrifuge preparation from leaf sap. Particles are on collodion film and are chromium-shadow-cast. ×54,000.

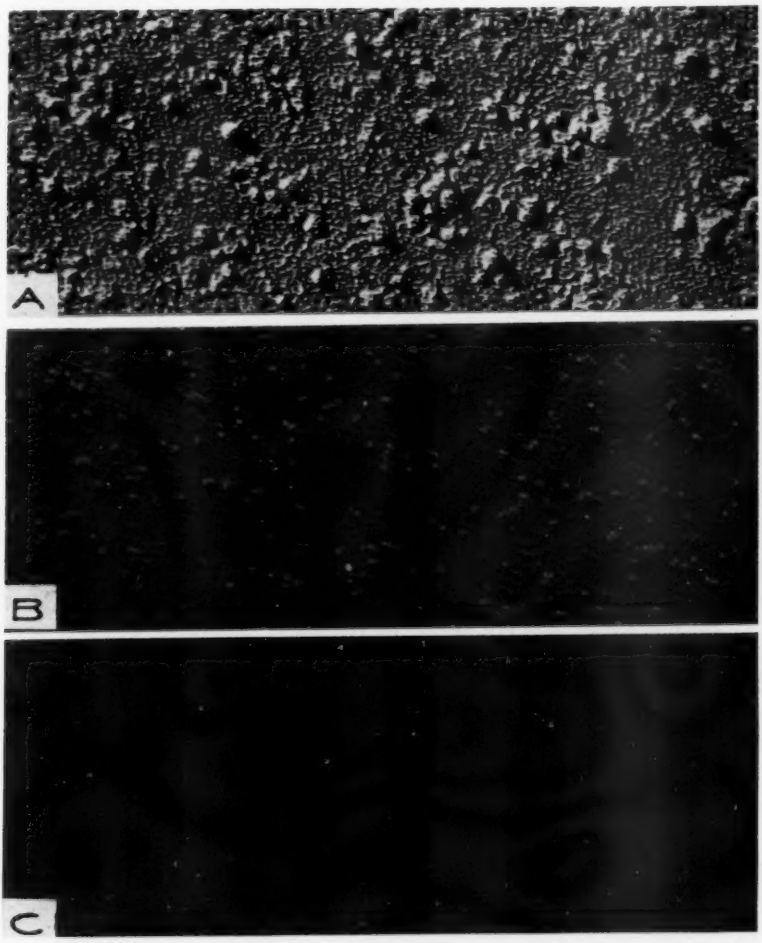
FIG. B. Toad-skin sap—prepared as above.

FIG. C. Normal plant sap—prepared by one cycle of ultracentrifugation, otherwise the same as above.

PLATE 9

PLATE 10

PLATE 11



THE RUSHES OF NORTH AND SOUTH CAROLINA

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Plates 12 to 14

This paper presents the results of a study to determine which species of the genus *Juncus* occur in North and South Carolina, as well as to assemble data on the habitats and distribution of these species within this area. This work is based mainly on material in the herbaria at Duke University, the University of North Carolina, North Carolina State College, the New York Botanical Garden and the Wiegand Herbarium at Cornell University. In all, some 1100 specimens from the two states have been examined. Since this number represents a fairly wide distribution of collections, it would seem that the species occurring in the Carolinas should be fairly well represented. However, more collections and field observations would no doubt add to the number of species as well as contribute more data regarding species variability, habitat relations and distribution.

Twenty-eight species have been found in the two states. New range extensions have been established for six species. One species, *J. trigonocarpus*, with a southern range, is reported here for the first time from North Carolina. Five others, *J. abortivus*, *J. diffussimuus*, *J. Longii*, *J. platyphyllus*, and *J. subcaudatus*, with northern ranges, are also reported for the first time from this state. Two more species, *J. Gerardii* Lois, and *J. Torreyi* Coville, the ranges of which include the Carolinas, may be expected to occur in this area, but apparently have not been collected here. One, *J. nodatus* L., which occurs in Virginia, may eventually be found in our area also.

Since time, original collections and field observations in this study were necessarily limited, no attempt has been made to work on infraspecific taxa.

In the Carolinas, as elsewhere, species of *Juncus* occur from the lowest to the highest altitudes. Some species, such as *J. effusus*, *J. acuminatus*, *J. marginatus*, and *J. tenuis*, are widely distributed. The last species shows a wide tolerance of soil conditions, occurring with about equal frequency in low, moist ground and dry, exposed situations. Because of its tendency to frequent ground packed by walking, it is often called "Path Rush." Examples of species found only at high altitudes are *J. trifidus* and *J. brevicaudatus*, which reach their southern range limits in the southern Appalachian Mountains. Several species are more or less confined to the Coastal Plain. The one which occurs closest to the coast is "Black Rush" (*J. Roemerianus*), which in more or less pure stands occupies extensive areas, especially on the borders of inlets and estuaries where the water table is affected by the tides, and the conditions are brackish. Examples of others which are typically Coastal Plain species are *J. abortivus*, *J. polycephalus*, and *J. megacephalus*. These species are adapted to moist habitats, such as stream, lake and pond margins, marshes, swamps, and roadside ditches. *J. megacephalus*, conspicuous by its large heads which turn dark with age, is often a characteristic

species of sand flats near the beach and between and back of the dunes. *J. polycephalus* and *J. repens* may be said to be truly amphibious. The most xerophytic species is *J. georgianus*, which occurs on exposed granitic rocks and reaches its northern range limit in the southeastern Piedmont of North Carolina.

MORPHOLOGICAL CHARACTERISTICS OF JUNCUS

The rushes compose a rather distinctive group of plants, as is evidenced by the frequently used term "rush-like." This refers to the cylindrical, scapose stems with reduced basal leaf blades characteristic of some of the common species. *Juncus* is easily distinguished from the genus *Luzula*, the only other North American representative of the family Juncaceae, in that the former is glabrous with open leaf sheaths and has a many-seeded, 1- or 3-celled capsule with parietal or axial placentae respectively, while the latter is hairy with closed sheaths and has a 3-seeded, 1-celled capsule with basal placenta.

A few of the rushes are annuals, but most of them are perennials with rootstocks, rootcrowns, or stolons. The scaly or woody rootstocks range from long and slender to short and stout or even bulbous. *J. nodosus* has tuber-bearing rootstocks while in *J. Elliottii* many of the fibrous roots end in fusiform tuberous structures.

Some species have leafy, erect or ascending stems which are terete or compressed, pithy or hollow, and rarely branched, occurring singly or in tufts. Other species are scapose. The leaf blades are terete, terete but channeled on the upper side, flat or grass-like. Distinct transverse septa are present in the terete or channeled blades of some species. In still others, the basal leaves are rudimentary or wanting. In some, the sheaths bear short or prolonged, entire or cleft auricles varying in texture.

The inflorescence is terminal, openly branched or occasionally congested (glomerate), or single-flowered. It is subtended by two involucral bracts, the lower of which is either well developed and leaf-like or, in scapose forms, appears as a continuation of the stem, resulting in an apparently lateral position of the inflorescence. In a few species the bracts are provided with conspicuous sheaths which make up an important part of the inflorescence. An example of this is *J. megacephalus*, in which the blades are obscurely developed or even wanting.

The flowers are comparatively small, regular, hypogynous and persistent. They are mostly anemophilous. The perianth consists of slightly differentiated calyx and corolla in trimerous whorls. The parts are glumaceous and inconspicuously colored. The stamens may be either 3 or 6 in number. If 3, they are borne opposite the sepals. The introrse anthers dehisce lengthwise. The pistil is 3-carpellate, with a slender style terminating in 3 filiform, minutely hairy style branches. The ovary may be 1-celled with parietal placentae or 3-celled with axial placentae, forming a loculicidial capsule with many seeds. The seeds are relatively small, spindle-shaped or broader, and distinctly ribbed and reticulated. In a few species the seed coats form characteristic tail-like appendages at the ends of the seeds. The time of flowering varies in different species from late spring to early autumn.

KEY TO SECTIONS AND SPECIES

1. Lowest involucral leaf erect, terete, appearing as a continuation of the scapose stem, the inflorescence therefore appearing lateral.
   2. Flowers bracteolate, i.e. subtended by two bracteoles in addition to the bracteole at the base of each pedicel, borne singly on the branches of the inflorescence; blades of basal leaves reduced to small vestiges or completely absent. Sect. GENUINI.
      3. Perianth as long as the capsule, the latter depressed at the tip, beakless or slightly mucronulate ..... 1. *J. effusus*
      3. Perianth about half as long as the capsule, the latter conical, pointed or mucronate ..... 2. *J. gymnocarpus*
   2. Flowers not bracteolate, i.e. subtended only by a single bracteole at the base of each pedicel, borne in clusters of 2–6, upper leaf sheaths bearing long, scape-like, pungent blades. Sect. THALASSII ..... 3. *J. Roemerianus*
1. Lowest involucral leaf not appearing as a continuation of the stem, or, if so, then not terete but channeled or flattened; the inflorescence usually appearing terminal; blades of basal leaf sheaths well developed.
   4. Leaf blades without septa.
      5. Flowers bracteolate, borne singly on the branches of the inflorescence. Sect. POIOPHYLLI.
         6. Annual with weak, fibrous roots; leaf sheaths without auricles ..... 4. *J. bufonius*
         6. Perennials with thickened rootstocks, stolons or tough fibrous roots; leaf sheaths auriculate.
            7. Leaf blades flat.
               8. Bracts longer than the inflorescence.
                  9. Auricles at summit of leaf sheaths large, thin, much prolonged beyond the point of insertion.
                     10. Several- to many-flowered; auricles entire ..... 5. *J. tenuis*
                     10. One- to 3-flowered; auricles deeply fringed ..... 6. *J. trifidus*
                  9. Auricles short and firm ..... 7. *J. platyphyllus*
               8. Bracts shorter than the inflorescence.
                  11. Perianth 2.5–3 mm. long; flowers chiefly on the upper side of the incurved branches (secund) ..... 8. *J. secundus*
                  11. Perianth longer, 4.5–6 mm.; flowers not secund ..... 9. *J. georgianus*
            7. Leaf blades terete, without septa.
               12. Capsule ellipsoidal; perianth parts not widely spreading ..... 10. *J. dichotomus*
               12. Capsule globose; perianth parts conspicuously spreading ..... 11. *J. coriaceus*
      5. Flowers not bracteolate, borne in glomerules of 2–12; leaf blades flat, grass-like. Sect. GRAMINIFOLII.
         13. Stems creeping or ascending; petals 6–10 mm. long; capsule linear; annual with weak, fibrous roots, often submerged ..... 12. *J. repens*
         13. Stems normally erect; petals 2-3 mm. long; capsule ovoid; perennials with rhizomes or stolons.
            14. Culms usually caespitose from stout, branching rootstocks.
               15. Glomerules few, rarely more than 30, 5–10-flowered; stamens shorter than sepals ..... 13. *J. marginatus*
               15. Glomerules many, 30 or more, 2–5-flowered; stamens longer than the sepals ..... 14. *J. biflorus*
            14. Culms usually solitary from long, slender rhizomes ..... 15. *J. Longii*
   4. Leaf blades septate. Sect. SEPTATI.
      16. Glomerules mostly single-flowered; flowers often aborted ..... 16. *J. abortivus*
      16. Glomerules several to many-flowered; flowers usually normally developed.
         17. Seeds with definite elongate, tail-like ends.

18. Plants of rigid texture; perianth spinescent, about half as long as the dark red, lustrous capsule ..... 17. *J. trigonocarpus*
18. Plants not rigid; perianth not spinescent; capsule not dark red.
19. Perianth about equalling the capusle; seeds conspicuously long-tailed 18. *J. canadensis*
19. Perianth distinctly shorter than the capsule; seeds short-tailed.
20. Inflorescence narrow, the branches strictly erect; glomerules few-flowered (3–7); capsule deep brown ..... 19. *J. brevicaudatus*
20. Inflorescence open, branches spreading; glomerules few- to many-flowered; capsule stramineous to brownish ... 20. *J. subcaudatus*
17. Seeds with merely pointed or blunt ends.
21. Flowers in dense spherical or hemispherical heads; capsule usually shorter than the perianth; or, if as long as, or longer than the perianth, then subulate.
22. Capsule less than three-fourths the length of the perianth, ovoid-conic 21. *J. brachycarpus*
22. Capsule equalling or exerted beyond the perianth, subulate.
23. Leaves flattened laterally ..... 22. *J. polycephalus*
23. Leaves terete.
24. Blade of uppermost leaf as long as or longer than its sheath; anthers exerted ..... 23. *J. scirpoides*
24. Blade of uppermost leaf much shorter than its sheath; anthers included ..... 24. *J. megacephalus*
21. Flowers not in dense spherical or hemispherical heads; capsule equal to or longer than the perianth, abruptly short-pointed.
25. Capsule 3.5–5 mm. long, twice the length of the perianth; cyme conspicuously diffuse ..... 25. *J. diffusissimus*
25. Capsule not more than 3.5 mm. long, less than twice the length of the perianth; cyme not especially diffuse.
26. Capsule 2–2.5 mm. long, dark brown; roots sometimes tuberiferous 26. *J. Elliottii*
26. Capsule 2.5–3.5 mm. long, straw colored; roots not tuberiferous.
27. Perianth equalling or slightly shorter than the capsule 27. *J. acuminatus*
27. Perianth only about two-thirds the length of the capsule 28. *J. debilis*

List of Species

In order to conserve space, the citation of specimens examined is confined to those taxa which are notably limited in distribution, and to those reported here for the first time from the Carolinas. The distribution of others is indicated in notes. Abbreviations for herbaria in which specimens are located are as follows:

DUKE Herbarium, Duke University
CLEMS Herbarium, Clemson College
CU Wiegand Herbarium, Cornell University
NCS Herbarium, North Carolina State College
NCU Herbarium, the University of North Carolina
NY Herbarium, the New York Botanical Garden

GENUINI (Buchenau) Vierh.

1. *J. effusus* L. Plate 12, Fig. 1.

One of the commonest and most widespread of our rushes, occurring from

the coast to the mountains. Moist to wet soil, edges of swamps, marshes, moist meadows, etc. Often conspicuous in pasture meadows where it is shunned by grazing animals. Specimens examined, 80. Throughout most of N. Amer. as far south as Mex.; also in Eurasia and eastern Asia.

2. *J. gymnocarpus* Coville. (*J. Smithii* Engelm, not Kunth). Plate 12, Fig. 2.
Local, in acid swamps, mountains of North Carolina: Burke Co., *Beaman 192* (NCS); Macon Co., *Wherry and Pennell 14157* (DUKE). Specimens examined, 2. Mountains of Tenn. and e. Pa.; also in Walten Co., Fla.

THALASSICI (Buchenau) Vierh.

3. *J. Roemerianus* Scheele. Plate 12, Fig. 3.
Coastal, in brackish marshes bordering inlets and estuaries where it frequently occupies vast areas in almost pure stands. Forms with compact inflorescences, similar to those of *J. effusus* L. var. *conglomeratus* (L.) Engelm., appear occasionally throughout populations of normal individuals. The significance of these abnormal forms in relation to the typical is not known. Specimens examined, 25. Fla. to Tex., n. to Md.

POIOPHYLLI (Buchenau) Vierh.

4. *J. bufonius* L. Plate 12, Fig. 4.
A weedy annual, occurring in moist soil in fields, on road shoulders and in ditches, most frequent in the Coastal Plain and extending into the lower Piedmont. Specimens examined, 25. Throughout N. Amer., Centr. Amer., the W. Ind., and S. Amer.; also in parts of the Old World.

5. *J. tenuis* Willd. (*J. macer* S. F. Gray). Plate 12, Fig. 6.
A common species throughout the area, in dry or moist soil as a component of the natural vegetation but more frequently in disturbed ground, especially in compacted soil as along roadsides and in paths. Specimens examined, 100. Widespread in N. Amer.; also in Eu. and n. Afr.

6. *J. trifidus* L. var. *monanthos* (Jacq.) Bluff and Fingerhuth. Plate 12, Fig. 7.
Local, at high elevations; represented by one collection from North Carolina: Buncombe Co., *Ashe 5546* (NCU). N. C. to e. N. Y. and N. H.

7. *J. platyphyllus* (Wieg.) Fern. (*J. dichotomus* var. Wieg.) Plate 12, Fig. 8.
In open, sandy soil, dry or moist situations. Apparently a Coastal Plain species but extending into the lower Piedmont. Not collected in South Carolina. North Carolina: Bertie Co, *Fox & Godfrey 1574* (NCS); Granville Co., *Blomquist 6169* (DUKE); Orange Co., *Ashe*, June, 1897 (NY); Person Co., *Batson 6* (DUKE); Wake Co., *Wells*, July 6, 1929 (NCS); Wilson Co., *Blomquist 14690* (DUKE). Me. s. to Fla.

8. *J. secundus* Beauv. Plate 12, Fig. 9.
In open, sandy or muddy soil. Not common. Collected only in the Piedmont of North Carolina: Durham Co. *Blomquist*, June 6, 1946 (DUKE); Gaston Co., *Ashe 84* (NCU); Granville Co., *Batson*, May 24, 1949 (DUKE);

Orange Co., *Ashe 1250b* (NCU); Person Co., *Ashe*, May 20, 1896 (NCU). N. C. to Me., w. to Ind., Tenn. and Mo.

9. *J. georgianus* Coville. Plate 13, Fig. 10.
In depressions in granitic outcrops, mostly from the lower Piedmont, North Carolina: Alexander Co., *Keever*, May 12, 1951 (DUKE); Franklin Co., *Batson*, May, 1950 (DUKE); Rowan Co., *Small*, Aug. 18–27, 1894 (NY); South Carolina: Lancaster Co., *Huntley 242* (DUKE). Piedmont, Ga. to N. C.

10. *J. dichotomus* Ell. Plate 13, Fig. 11.
A common and widely distributed species in the Carolinas, occurring in dry or moist sandy soil in various situations except in the higher mountains. Specimens examined, 85. Coastal Plain and adjacent areas, Fla. to Tex. and Mex., n. to Mass.

11. *J. coriaceus* Mackenz. (*J. setaceus* of authors, not Rostk.) Plate 13, Fig. 12.
Swamps, marshes, edges of ponds, lakes and streams and roadside ditches. A common species throughout the Coastal Plain extending into the lower Piedmont. Specimens examined, 85. Fla. to e. Tex., n. to Ark., Okla. and N. J.

GRAMINIFOLII—Buchenau

12. *J. repens* Michx. Plate 12, Fig. 5.
Muddy shores and often submerged in shallow water in ponds, lakes, ditches and streams; when not flowering, simulating certain other aquatics. Common in the Coastal Plain, extending into the lower Piedmont. Specimens examined, 30. Fla. to Tex., n. to Ark., Okla., Tenn. and Del.

13. *J. marginatus* Rostk. Plate 13, Fig. 13.
Moist, sandy or peaty soil, various situations, throughout the Carolinas. Specimens examined, 48. Fla to Tex., n. to Mich. and Me.

14. *J. biflorus* Ell. (*J. aristulatus* of authors, not Michx.) Plate 13, Fig. 14.
Moist to wet soil, meadows, borders of ponds, lakes and streams. The most common rush in roadside ditches of the Coastal Plain. Variable in its inflorescence; an occasional form with congested glomerules has been called f. *adinus* Fern. & Grisc. Throughout the Coastal Plain of the Carolinas, extending into some of the lower Piedmont counties. Specimens examined, 130. Fla. to Tex., n. to Mo., Ill., Mich., N. Y. and Mass.

15. *J. Longii* Fern. Plate 13, Fig. 15.
Apparently infrequent, Coastal Plain, rarely in Piedmont. Similar to *J. biflorus* but with elongate, slender rhizomes. Not collected in South Carolina. North Carolina: Harnett Co., *Godfrey & Fox 49415* (DUKE, NCS); Iredell Co., *Keever 369* (DUKE). N. C. to N. J.; La. to Mo. and Okla.

16. *J. abortivus* Chapm. Plate 13, Fig. 16.
Easily recognized by its terete leaves, diffuse filiform panicles with scattered, mostly single-flowered glomerules. Moist pine barrens and shores.

Lower Coastal Plain. Specimens examined, 23. North Carolina: Beaufort Co., *Wells*, Oct. 5, 1923 (NCU); Bladen Co., *Woodbury 185* (DUKE); Carteret Co., *Blomquist 14736* (DUKE); New Hanover Co., *Curtis*, 1893 (NY); South Carolina; Darlington Co., *Coker*, Aug. 1908 (NCU). Fla. to Va.

17. *J. trigonocarpus* Steud. Plate 13, Fig. 17.
A southern species reaching its northern range limit in North Carolina. Sandy bogs, Coastal Plain. Specimens examined, 8. North Carolina: Harnett Co., *Godfrey 50120* (DUKE, NCS); Moore Co., *Godfrey 50723* (DUKE, NCS); South Carolina: Aiken Co., *Ravenel*, Sept. 6–Oct. 6, 1866 (NY). Fla. to La., n. to N. C.

18. *J. canadensis* J. Gay. Plate 13, Fig. 18.
A late blooming species. Low acid marshy ground, mainly in the Coastal Plain, extending into the lower Piedmont. Specimens examined, 36. Ga. to La., n. to Minn., Me. and e. Canada.

19. *J. brevicaudatus* (Engelm.) Fern. Plate 14, Fig. 19.
Wet places at higher elevations in the mountains. Specimen examined, 1. North Carolina: Yancey Co., *Clausen & Trapido*, 1936 (CLEMS). At lower elevations northward to Minn., Ill., Mich., Me. and e. Canada.

20. *J. subcaudatus* (Engelm.) Coville & Blake.
Cool, moist, shaded places in the mountains. Specimens examined, 2. North Carolina: Macon Co., *Wherry & Pennell 14158* (DUKE); Jackson Co., *Godfrey & Fox 49928* (DUKE). Ga. to Mo., n. to W. Va., N. Y. and Mass.

21. *J. brachycarpus* Engelm. Plate 14, Fig. 20.
Moist, sandy soil, mostly from the Piedmont. Specimens examined, 20. S. C. to Tex., n. to Va. and locally to Mo., Okla., Ill., Ind., Mich., Mass., and e. Canada.

22. *J. polycephalus* Michx. Plate 14, Fig. 21.
A robust species with laterally compressed, sword-shaped leaves and numerous large heads. Sandy pond margins, moist savannas and ditches, lower Coastal Plain. Specimens examined, 25. Fla. to La., n. to N. C.

23. *J. scirpoides* Lam. Plate 14, Fig. 22.
Moist to wet soil, various situations, marshes, borders of swamps, moist savannas, meadows and roadside ditches, Coastal Plain, extending into the lower Piedmont. Specimens examined, 90. An occasional form with lobulate heads ranging from Fla. to Long Island has been segregated as var. *meridionalis* Buchenau. Fla. to Tex., n. to Okla., Ill., Ind., Mich., N. Y., N. J.

24. *J. megacephalus* M. A. Curtis. Plate 14, Fig. 23.
A robust species with conspicuously large heads which persist and turn dark with age. Brackish to fresh marshes near the coast, often seen in depressions between and back of the dunes. Specimens examined, 30. Fla. to La., n. to e. Va.

25. *J. diffusissimus* Buckley. Plate 14, Fig. 24.

As the name suggests, this species is characterized by conspicuously diffuse pannicles, loosely dichotomously branched. Wet, clayey or sandy soil, most frequent in the Coastal Plain but local in the Piedmont and mountains. Specimens examined, 11. North Carolina: Person Co., *Batson 4* (DUKE); Columbus Co., *Batson*, May, 1950 (DUKE); Jackson Co., *Godfrey & O'Connell 51508* (DUKE, NCS); South Carolina: Berkley Co., *Wiegand & Manning 752* (CU); Charleston Co., *Moldenke 1215* (DUKE); Dorchester Co., *Correll 5331* (DUKE); Florence Co., *Wiegand & Manning 751* (CU); Williamsburg Co., *Godfrey & Tryon 406* (DUKE). Ga. to Tex., n. to Kans., Ind. and Va.

26. *J. Elliottii* Chapm. Plate 14, Fig. 25.

An interesting species in that many of its roots end in fusiform "tubers". Mostly in wet soil, margins of bogs, ponds, and in shallow ditches, often partly submerged. Lower Coastal Plain. Specimens examined, 31. Fla. to Tex., n. to Del.

27. *J. acuminatus* Michx. Plate 14, Fig. 26.

Slender culms with about 2 leaves, named from its acuminate, pointed sepals and petals. Heads often proliferating. Moist soil in various situations throughout the area from the coast to the mountains. Specimens examined, 105. Ga. to Tex. and Mex., Minn., Wisc., New Eng. and Nova Scotia.

28. *J. debilis* A. Gray. Plate 14, Fig. 27.

Similar to *J. acuminatus* but with weaker stems and flaccid leaves. Wet places, margins of ponds and streams and in ditches. Coastal Plain, extending into the lower Piedmont. Specimens examined, 35. Fla. to Ala., n. to Mo. and Conn.

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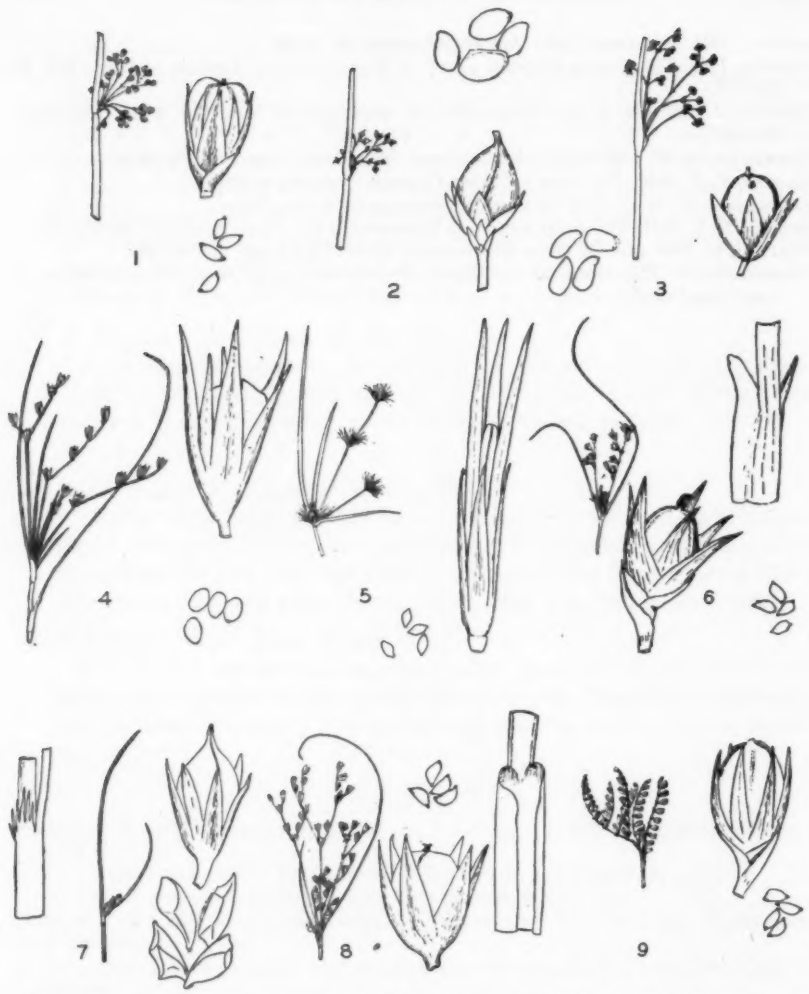
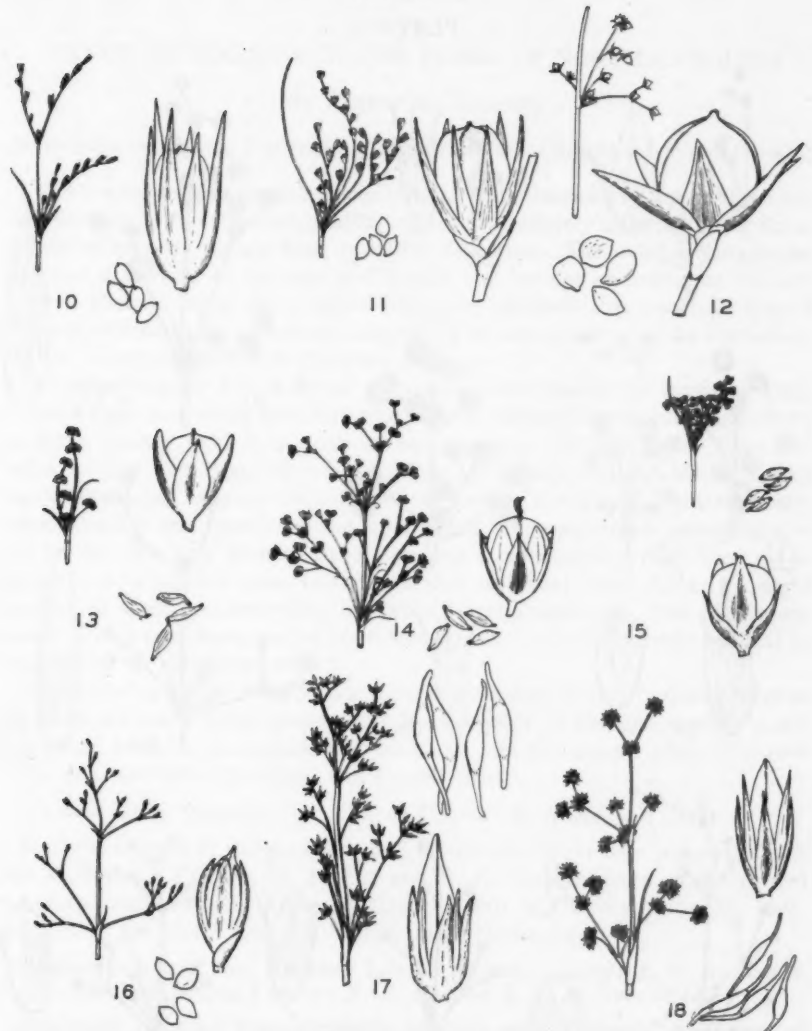
PLATE 12



1. *J. effusus*. 2. *J. gymnocarpus*. 3. *J. Roemerianus*. 4. *J. bufonius*. 5. *J. repens*. 6. *J. tenuis*. 7. *J. trifidus*. 8. *J. platyphyllus*. 9. *J. secundus*.

PLATE 13



10. *J. georgianus*. 11. *J. dichotomus*. 12. *J. coriaceus*. 13. *J. marginatus*. 14. *J. biflorus*. 15. *J. Longii*. 16. *J. abortivus*. 17. *J. trigonocarpus*. 18. *J. canadensis*.

PLATE 14

19. *J. brevicaudatus*. 20. *J. brachycarpus*. 21. *J. polycephalus*. 22. *J. scirpoides*. 23. *J. megacephalus*. 24. *J. diffusissimus*. 25. *J. Elliottii*. 26. *J. acuminatus*. 27. *J. debilis*.

RANGE EXTENSIONS IN THE FLORA OF NORTH CAROLINA

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This is a report of range extensions into the state, extensions from one province into another within the state, and records of plants rarely collected in the state. These records are mostly from my 1951 collections. The cited specimens are grouped according to habitat, and within the habitat according to Fernald (1950). General range data, unless otherwise specified, are also from Gray's Manual of Botany. All specimens collected by me are deposited in the Herbarium of the University of North Carolina.

While looking for lime sinks on the coastal plain during the spring of 1951, I found some interesting marl outcrops high in calcium content on Island Creek in Jones County, and on a small stream emptying into the Neuse River two miles north of Fort Barnwell in Craven County. In both localities the calcareous marls outcropped only on the bends of the creeks. The Island Creek exposures were extensive and numerous; the outcrops on the small stream emptying into the Neuse were very limited. In no case was a marl outcrop more than a few square feet in exposed area. In the meanders of Island Creek a marly loam is present on which an interesting hardwood forest is maturing. The Pleistocene sands in this area have eroded considerably and the soil is largely residual in origin from the calcareous marl.

The following list represents collections of specimens of distributional interest found on the marl. These species, with the exception of *Rorippa sessiliflora*, are extensions from the mountains and piedmont into the coastal plain. The fern range data are from Blomquist and Correll (1940).

Woodsia obtusa (Spreng) Torr. Craven County: *A. E. Radford 5723.*

Fairly abundant in the piedmont and mountains. Previously reported as far east as Franklin County. W. B. Fox and R. K. Godfrey have an unrecorded collection from Johnston County, 2 miles north of Clayton, May 29, 1948, no. 1682 in the North Carolina State College Herbarium.

Cystopteris fragilis var. *Mackayii* Lawson. Craven County: *A. E. Radford & J. A. Duke 5618;* Jones County: *A. E. Radford & J. A. Duke 5645.*

Previously recorded from mountain counties only. Recently specimens of *Cystopteris fragilis* var. *protrusa* Weath. have been collected along the Deep River in the piedmont by W. B. Fox and R. K. Godfrey, no. 49377, North Carolina State College Herbarium, and by A. E. Radford, no. 5105.

Asplenium resiliens Kuntze. Craven County: *A. E. Radford & B. M. Taylor, Jr. 5523; A. E. Radford & J. A. Duke 5608;* Jones County: Reedy Creek, *A. E. Radford & J. A. Duke 5639; A. E. Radford & J. A. Duke 5722.*

Rarely collected in North Carolina. Recorded as far east as Yadkin and

Stanley Counties in the piedmont. Other collections have been made in the mountains.

Pelleae atropurpurea (L.) Link. Jones County: *A. E. Radford & J. A. Duke 5645.*

Rarely found in this state. Known as far east as the Blue Ridge Escarpment in McDowell County.

Liparis lilifolia (L.) Richard. Jones County: *A. E. Radford 5726.*

Reported as far east as Durham County in the piedmont by Correll (1937)

Asarum canadense var. *reflexum* (Bickn.) Robins. Craven County: *A. E. Radford & B. M. Taylor, Jr. 5528.*

Ranges from Ct. to s.e. Man., s. to N.C. The easternmost specimen in the state herbaria is from Durham County. A specimen collected by L. E. Anderson, no. 6277, May 2, 1938, is in the Duke University Herbarium.

Rorippa sessiliflora (Nutt.) Hitchc. Craven County: *A. E. Radford & J. A. Duke 5611.*

Ranges from n.w. Fla. to Tex., n. to Ind., Wisc., Minn., and Neb.; Potomac River, D.C.; James River, e. Va. Apparently this is the first record from North Carolina.

Waldsteinia fragarioides var. *parviflora* (Small) Fern. Jones County: *A. E. Radford 5725.*

No specimen of this fairly common mountain and piedmont plant was in the state herbaria from the coastal plain.

Some time was spent this past year in studying an herbaceous community with mountain and northern affinities on the wooded floodplains of the Haw, Deep, and Cape Fear Rivers. The community in most cases consisted of extensive colonies of *Asarum canadense* var. *reflexum* (Bickn.) Robins., *Claytonia virginica* L., *Corydalis flavula* (Raf.) DC., *Cystopteris fragilis* var. *protrusa* Weath., *Dentaria diphylla* Michx., *Dicentra Cucullaria* (L.) Bernh., *Hybanthus concolor* (T.F. Forst.) Spreng, *Nemophila microcalyx* (Nutt.) F. & M., *Osmorhiza longistylis* (Torr.) DC., *Phacelia ranunculacea* (Nutt.) Constance, and *Viola pubescens* Ait.

The species of the genera listed were restricted to the alluvium on the northern slopes, except where the surrounding rock was basic. Between Central Falls and Worthville on the Deep River in Randolph County most of the alluvium is derived from the abundant quartz in the area. There the characteristic alluvial vegetation is missing except where a diabase dike intersects the river one fourth mile south of Worthville.

Some of the more interesting species from a geographic standpoint are the following:

Isopyrum biternatum (Raf.) T. & G. Lee County: *A. E. Radford, M. B. Huneycutt & J. A. Duke 5551.*

Occurs in rich or calcareous woods, s. Ont. to Minn., s. to n.w. Fla., Ala. Mo., and Tex. This is the second record from North Carolina. Fox, Godfrey, and Blomquist (1950) have reported it previously.

Hydrophyllum virginianum L. Lee County: *A. E. Radford, M. B. Huneycutt & J. A. Duke 5555;* Wake County: Richland Creek near its junction with Crabtree Creek, n.w. of Raleigh, May 16, 1950, *R. K. Godfrey & Herman Wiebe 50384.*

Ranges from Que. to Man., S. through W. N.E. to Va. and to Tenn., N. Ark. and E. Kan. *Hydrophyllum virginianum* var. *atranthum* (E. J. Alex.) Constance has been reported from the mountains of western North Carolina. These collections represent the first specimens of the species from the piedmont of North Carolina. Some doubt has arisen as to whether the variety is a good segregate of a rather variable species. Duplicates of specimens of Hydrophyllaceae and Umbelliferae reported in this paper have been determined or authenticated by Lincoln Constance.

Nemophila microcalyx (Nutt.) Fisch. & Mey. Moore County: *A. E. Radford 5512;* Lee County: *A. E. Radford, M. B. Huneycutt & J. A. Duke 5550;* Alamance County: *A. E. Radford & W. Hight 5569;* Bladen County: *A. E. Radford 5705.*

Ranges from w. Fla., Tex., n. to e. Va., Tenn., Mo., Ark. and Oklahoma. Not reported by Blomquist and Oosting (1948). I have not seen any specimens in the state herbaria. This is one of the most abundant species on the alluvium.

Phacelia ranunculacea (Nutt.) Constance. Alamance County: *A. E. Radford & W. Hight 5570;* Lee County: *A. E. Radford, M. B. Huneycutt & J. A. Duke 5590.*

Occurs in rich woods and on alluvium, upper Potomac River, Md., D.C., and Va. These records represent a southward extension into North Carolina. The species was abundant where found.

Several new range extensions of vascular aquatic plants were made from collections in the coastal region of the state:

Isoetes saccharatum Engelm. Currituck County: *A. E. Radford 5766;* Craven County: *A. E. Radford & M. B. Huneycutt 5848.*

Previously recorded only from Tyrrell County in North Carolina by Radford (1950). This is a southward extension into the central coastal region.

Lilaeopsis carolinensis C. &. R. Currituck County: *A. E. Radford 5753; A. E. Radford 5772;* Dare County: *A. E. Radford 5764.*

Recorded from five localities in the U.S., including two from North Carolina by Rodgers (1950). My three collections are from new localities.

Limosella subulata Ives. Currituck County: *A. E. Radford 5756.*

The range of this small aquatic is extended south from the Chesapeake Bay area into North Carolina. Duplicate authenticated by F. W. Pennell.

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A NEW WATER MOLD ON KERATINIZED MATERIALS

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Plate 15

In a soil sample collected from a hog lot in 1948 a fungus was found with asexual reproduction similar to that in the genus *Leptolegnia* and with resting bodies somewhat resembling those of *Lagenidium* or *Aphanomycopsis*. Human hair was used as bait and as a substratum for culturing the fungus, according to the methods first used by Karling (1946). On hair it developed an intramatrical mycelium which in places almost filled the hair. The sporangia formed within the hair, the spores emerging through rather long exit tubes. The resting bodies were observed in large quantities within the hairs. Difficulty was encountered in culturing this isolate and it was lost before it could be studied thoroughly. Since early summer of 1950, many soil collections have been made from barnyards, pastures, chicken lots, etc., in an effort to obtain this fungus as well as other fungi which occur on keratinized materials. For bait, human hair, human skin, thin shavings of cow horn, horse hoofs, and chicken and turkey feathers have been used (Karling 1946). In seventeen of these collections this peculiar fungus has been observed. Six isolates have been maintained in unifungal culture for several months. Two of these have been maintained in pure culture since September, 1951.

In unifungal and pure culture work, cow horn shavings, human hair and skin have been used almost entirely. Attempts to culture this fungus on these substrates in charcoal-treated tap water resulted in failure. It was found to be relatively easy, however, to grow the isolates on human skin, cow horn, etc., in sterilized soil water. The technique for making soil water extract was a modification of Bold's. It was made by autoclaving 500 grams of soil, containing much organic matter, in 1000 ml. of tap water for one hour. This was filtered and enough tap water added to the filtrate to make one liter. Pure cultures have been grown on an agar made by adding two grams of cow horn filings to one hundred milliliters of a 1:7 dilution of the soil extract. One per cent agar has been used for the most part. Some growth of the isolate was obtained on 1.5% agar containing 15 grams of maltose and 0.04 grams of meat peptone per liter. Further pure culture studies are now in progress. Attempts to grow it on grass leaves (Paspalum sp.) and hemp seed have failed.

Since this fungus does not seem to fit in any existing genus, it is described as a new one.

Leptolegniella gen. nov.

Hyphae intramatricales irregulares ramosae aliquando septatae, rhizoideis gracilibus ramosis partim zoosporas formantes. Zoosporae ut *Leptolegnia* exuntes immaturae et encystantes. Sporae perdurantes asexuales in hyphis restantes.

Mycelium developing mostly intramatrically, hyphae very irregular, much

branched, occasionally septate. Rhizoids slender and branching. Zoosporangia branched, not differentiated from the vegetative mycelium. Zoospores emerging incompletely formed, as in *Leptolegnia*, diplanetic. Resting bodies formed within the hyphae by rounding up of portions of the protoplasm, asexually formed.

With chloro-iodide of zinc the old hyphal walls give a reddish-purple reaction.

Leptolegniella keratinophilum sp. nov.

Fungus saprophyticus keratinicolus. Hyphae ramosae intramatricales plerumque 5–20(–25)μ crassae, rhizoideis ramosis gracilibus apicibus hebetibus. Zoosporangia ramosa, in tubulos ad 395μ longosplerumque 5–13.5(–18.5)μ latos exeuntes. Zoosporae biflagellatae Leptolegniis similes encystantes; cystis 5.4–7.2 (raro –13)μ diam. Sporae perdurantes in hyphis restantes, sphaericae 9–12(–23)μ diam. vel ovoideae 10.8–16.7μ × 6.5–10.7μ, aliquando irregulares, pariete levi 0.5–2μ crasso; corpus granulosum centrale sphericum vel subsphericum 5.4–6(–11)μ diam., globulo refractivo centrico vel excentrico 3.5–4(–5.8)μ diam.

Mycelium mostly intramatrical on keratinized materials. Hyphae irregularly and much branched, usually from 5 to 20μ in diameter, sometimes to 25μ. Rhizoids formed inside and outside the substratum, slender, branched, tapering to a blunt tip. Zoosporangia branched, with straight to sinuous exit tubes up to 395μ in length, and usually from 5 to 13.5μ in width, sometimes to 18.5μ. Zoospores elongate upon emergence, bending as in *Leptolegnia*, becoming pip-shaped with two apical flagella; diplanetic, usually 5.4 to 7.2μ in diameter in the encysted state, rarely to 13μ. Resting bodies formed within the unswollen hyphae by rounding up of portions of the protoplasm, spherical to subspherical, usually 9 to 12μ in diameter, sometimes to 23μ, ovoid, usually from 10.8 to 16.7μ in length by 6.4 to 10.7μ wide, sometimes irregular in shape. Wall of resting body evenly thickened, from 0.5 to 2μ thick. Granular central body spherical to subspherical, about 5.4 to 6μ in diameter, sometimes to 11μ. Refractive globule centric to eccentric, about 3.5 to 4μ in diameter, sometimes to 5.8μ.

Saprophytic on keratinized materials, from a sheep pasture in Wilson County, Tennessee.

This fungus appears to be related to *Leptolegnia*. In the latter an extensive extramatrical mycelium is produced, while in the present fungus the mycelium is almost entirely intramatrical. In *Leptolegnia*, sex organs are produced, whereas in this fungus the resting bodies are formed within undifferentiated hyphae.

Because of its thinness and relative transparency, human skin was found to be the most favorable substrate on which to make detailed observations of this fungus. On skin it develops rapidly, usually making it possible to observe zoospore discharge within 48 hours after inoculation. Most observations have been made using hanging drop mounts, since a cover glass hinders spore discharge. Observations were made with a 97× oil immersion objective and a 10× ocular.

The mycelium within the skin branches profusely, becoming so intertwined that one gets the impression that anastomosis occurs. The zoosporangia are undifferentiated parts of the mycelium; thus the sporangia are branched. Zoospore cleavage has been observed on several occasions. It occurs as in members of the Saprolegniaceae. In the thinner hyphae and in the exit tubes the spores are in single rows. In thicker parts of the hyphae the spores may be in two rows.

The spores emerge elongate, rod-like and assume their mature form out in the water in the peculiar manner of the zoospores of *Leptolegnia*, as first de-

scribed by Coker in 1909. Upon becoming anteriorly biflagellate they swim with sluggish movement. Some of the spores emerging last from a sporangium may be almost or completely formed. Usually these spores encyst quickly, later emerge and become laterally biflagellate as in the second swimming stage of *Leptolegnia*. Thus the spores are diplanetic. In some instances the spores which had encysted in an exit tube were seen to germinate by germ tubes.

Resting body formation occurs by the cleavage and rounding up of large portions of the protoplasm. At first the protoplasm is very granular. Gradually there is formed in the center of each resting body a spherical to subspherical granular body and within this is a refractive, spherical globule. Whether or not nuclear fusion occurs is not known. This must await cytological study.

SUMMARY

A new fungus is described on keratinized materials. It is characterized by a poorly developed extramatrical and an extensive intramatrical mycelium, and asexual reproduction much as in *Leptolegnia*. Sex organs are not produced, the resting bodies forming within undifferentiated hyphae. The new fungus is named *Leptolegniella keratinophilum*.

ACKNOWLEDGMENTS

The author is indebted to Dr. J. N. Couch under whose guidance this work was done, to Dr. C. E. Wood for the Latin descriptions, and to Miss E. Kathleen Goldie-Smith for suggestions pertaining to the arrangement of figures.

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PLATE 15

Drawings were made with the aid of camera lucida. All figures × 740.

FIG. 1. Part of a zoosporangium with emerging zoospores. The zoosporangium is intramatrical except for the exit tube.

FIG. 2. Zoospores in various stages of formation after emergence.

FIG. 3. An encysted spore and a laterally biflagellate zoospore which has emerged from the cyst.

FIG. 4. Part of a zoosporangium before zoospore emergence. The zoosporangium is intramatrical except for the exit tube.

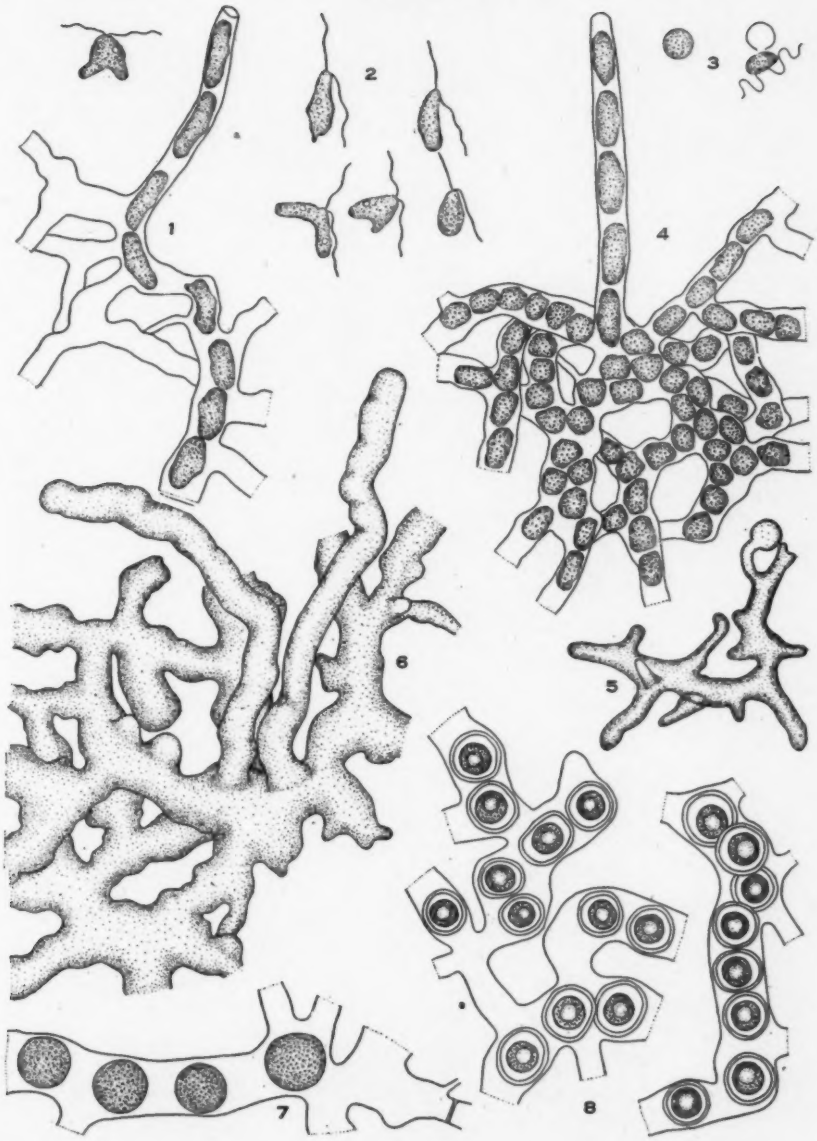
FIG. 5. Young thallus and the spore from which it grew, on the edge of a piece of human skin.

FIG. 6. Part of much-branched mycelium with two exit tubes formed.

FIG. 7. Four immature resting bodies within a hypha. This figure also shows a septum.

FIG. 8. Mature resting bodies within the hyphae.

PLATE 15



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